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AUTHOR – AUTEUR

Full Name of Author – Nom complet de l'auteur

Glenn D. Harrison

Date of Birth – Date de naissance

Sept 14, 1952

Canadian Citizen – Citoyen canadien

☒ Yes / Oui ☐ No / Non

Country of Birth – Lieu de naissance

CANADA

Permanent Address – Résidence fixe

11013 University Ave.
Edmonton, ALTA. T6G 1Y4.

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Name of Supervisor – Nom du directeur de thèse

Dr. J. Addicott

AUTHORIZATION – AUTORISATION

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by

Glenn D. Harrison

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(SIGNED) Glenn Harrison

PERMANENT ADDRESS:

11013 University Ave

Edmonton Alberta

T6G 1Y7

DATED Oct 4 1985

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Host plant discrimination and the evolution of feeding preference in the Colorado potato beetle *Leptinotarsa decemlineata* (Say) submitted by Glenn D. Harrison in partial fulfilment of the requirements for the degree of Master of Science.

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Supervisor

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Date 26 April 1985

Abstract

Variations in plant sampling and feeding behaviour were used to examine mechanisms of host plant discrimination and the evolution of feeding preference in the Colorado potato beetle *Leptinotarsa decemlineata*. Beetles from three geographic populations were videotaped on four host plants, representing the three regional hosts and ancestral host of the species. Beetles were also tested on leaves treated with solanaceous alkaloids to determine whether these compounds act as sensory inhibitors of feeding.

On average, duration of sampling on the plant surface prior to biting systematically increased on plant species less preferred for feeding, indicating beetles were capable of discriminating among plant species based on olfaction and/or contact chemoreception. Sampling of plant fluids (gustatory sampling) also increased on less preferred species for those beetles that proceeded to feed. Movements of the antennae during gustatory sampling suggest one of the functions of this behaviour is to enhance the release of plant volatiles.

Host plants of *L. decemlineata* contain steroidal glycoalkaloids. The glycoalkaloids tomatine (from tomato) and solanine (from potato) did not affect first meal size or duration of plant sampling when tested at concentrations exceeding natural levels. These compounds are apparently not responsible for determining different patterns of initial host acceptance within regional populations of beetles.

Tropane alkaloids are found in solanaceous plants not used by *L. decemlineata* as hosts. When incorporated into potato, the tropane alkaloid, atropine, reduced meal size and increased gustatory sampling behaviour by beetles. However, tropane alkaloids may not restrict the beetles' host range, since plants containing these compounds may be discriminated against on the basis of olfaction before plant fluids containing the alkaloids are sampled.

Regional populations of *L. decemlineata* that have colonized new plants have not lost their preference for ancestral hosts. Relative to physiological adaptations, behavioural adaptations leading to acceptance of novel host plants may have developed slowly during colonization events. Other studies on insect herbivores are reviewed which support this

pattern. I conclude that for some insect species, host selection behaviour may represent a conservative force in insect-plant evolution. For these species, local selection pressures favoring altered patterns of host use may be the primary cause of host shifts.

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I. Introduction

The vast majority of phytophagous insect species restrict their host use to one plant species (monophagy) or a few related plant species (oligophagy). Within wide ranging species of insects especially, further specialization may lead to the formation of host adapted races or biotypes. Possible reasons for this specialization include the diversity and distribution patterns of host plants (Price 1980, and references therein), plant chemical defenses against herbivory (Feeny 1975, Rhoades and Cates 1976), and community properties such as competition or the local presence of predators or parasites (Fox and Morrow 1981). Host specialization may promote rapid speciation and has probably been a major factor in contributing to the enormous diversity of insect herbivores (Mayr 1963).

Studies of phytophagous insects have long been prominent in the evolutionary literature, and there are currently several active debates in this field of study. One controversy centers around claims that some species or host races have evolved under sympatric conditions (Bush 1969, 1975a, Guttman et al. 1981; but see Futuyma and Mayer 1980 for an opposing viewpoint). Another controversy concerns the extent to which individual insect and plant taxa have coevolved (engaged in adaptation and counter-adaptation through evolutionary time). Interest in this idea grew after publications by Fraenkel (1959) and Ehrlich and Raven (1964) which related host specificity and patterns of insect evolution to the defensive properties of secondary plant compounds. Recently however, the probability of strict reciprocal coevolution between insects and plants has been questioned (Futuyma 1983, Jermy 1984), as has the assumption that herbivore specialization is ordinarily a response to secondary plant chemistry (Fox and Morrow 1981).

Resolving questions about insect-plant evolution is often hampered by a poor understanding of host selection behaviour in insects, the mechanisms which control it, and their genetic basis. In the absence of real data, assumptions are often made regarding the flexibility of insect behaviour and its role in mediating host shifts. For example, most models of sympatric speciation assume that mutations at a single locus can lead to changes in

host use (see Bush 1975b). Many of these assumptions are based on the notion that interactions between insects and plants are based on simple processes. However, mechanisms regulating host selection in insects are considerably more complex than first suspected. Traditional concepts that stressed single compound control of host choice have failed to account for the accumulated evidence, and new theories are gaining acceptance which emphasize the integration of total sensory input and the interaction of both pre- and post-ingestive mechanisms (Dethier 1982, review by Miller and Strickler 1984).

The present study was designed to clarify the role of behaviour in insect-plant evolution through analysis of evolutionary changes in the mechanisms which control host acceptability for feeding in the Colorado potato beetle *Leptinotarsa decemlineata* (Say). This oligophagous insect has recently undergone a host range expansion in North America induced by agricultural practices and therefore provides an opportunity to study behavioural phenomena associated with changes in host plant use. Adult food choice largely directs oviposition sites in this species, since females generally lay eggs where they settle to feed and do not fly when gravid (see Chapter four).

This thesis is organized around three chapters. Chapter two provides a detailed behavioural analysis of plant sampling and feeding behaviour and examines how beetles discriminate among potential host plants. The approach taken in this study is neuroethological, and is based on the rationale that mechanisms controlling host choice can be studied indirectly through variations in the insect's own behaviour patterns. As Fentress (1980) has stated, "Nervous systems do not simply build up a whole pattern through blind summation of the parts; they insist on the whole and adjust the parts accordingly." My objective was to identify "parts" of the the feeding sequence in beetles that vary in relation to plant preference, and use these relationships to suggest what mechanisms are responsible for allowing discrimination among plants. I also compare the behaviour of three recently evolved, host adapted populations of *L. decemlineata*, to determine how rapidly mechanisms controlling host plant recognition have evolved in this insect species.

Chapter three describes tests of the hypothesis that solanaceous alkaloids (secondary plant compounds) act as sensory inhibitors of feeding, and thereby function to regulate host choice in *L. decemlineata*. This possibility has been suggested from long term feeding studies, but these studies have not directly addressed mechanisms. In my experiments, variations in pre-ingestive sampling and feeding were used to directly observe the effects of alkaloids on behaviour.

In the final chapter, I review previous experiments on *L. decemlineata* and compare the behavioural and physiological adaptations that have evolved in this species during the formation of regional biotypes. I also review other studies in which both physiological and behavioural traits have been measured on the same insect species, in an attempt to assess the relative contribution of these adaptations in mediating host transfers.

Study Organisms

Leptinotarsa decemlineata is an oligophagous beetle which feeds exclusively on members of the Solanaceae, and primarily on plants of the genus *Solanum*. According to Tower (1906), *L. decemlineata* is descendant from *L. intermedia* Tower, an insect indigenous to Mexico where it feeds on Buffalo bur, *Solanum rostratum* Dunal. Tower suggests that movements of Spanish caravans into the United States in the seventeenth century allowed the northward expansion of both *S. rostratum* (the burs of the plant being carried by pack animals) and the insect. Sometime later *L. decemlineata* is presumed to have evolved from *L. intermedia* along the eastern foothills of the Rocky Mountains. However, in a revision of the genus Jacques (1972) regarded the two species as synonomous, and this treatment is now generally accepted.

During the mid-nineteenth century, potato (*Solanum tuberosum* L.) was introduced to the midwestern United States. *L. decemlineata* soon colonized potato and later dispersed across North America and Europe with the spread of potato agriculture (see Johnson 1969). Previous expansion of the beetles' range to the north or east had been prevented by the

limited distribution of its primary *Solanum* hosts (see Fig. 1.1). *L. decemlineata* is now reported to feed on a number of wild and domestic solanaceous plants.

L. decemlineata reproduces by sex; there is no parthenogenesis in the species. Eggs are laid by females directly on the plant and larvae develop through four instars before burrowing into the soil to pupate. Depending on latitude, there may be from one to three generations per year. Although relatively large, adult beetles are reported to be good fliers and dispersal by flight may be common, especially where host availability is limited (Johnson 1969).

Collection sites for the three regional populations of *L. decemlineata* used in this study, and approximate distributions of the two primary wild host plants of the species are shown in Figure 1.1. In Arizona, beetles are isolated on one host plant, *Solanum elaeagnifolium* Cav., a perennial weed common along roadsides and other disturbed areas. In this region, however, beetles are rare and are normally found only in irrigated valleys, perhaps where moist soil conditions permit successful pupation and adult diapause. Tower (1906) made similar observations of the species' distribution in Mexico, noting that although *S. rostratum* grew on the open plains, beetles were only common near stream beds or other sources of water.

Potato, an occasional winter crop in Arizona, is grown only during the beetles natural diapause period (November to April) and is therefore not used as a host. During a week long search for the insects in southeastern Arizona in September 1983, I found no other *Solanum* species in the area, an observation consistent with those of Hsiao (1978) and the herbarium records at the University of Arizona in Tucson. Based on the records contained in Tower (1906), *L. decemlineata* has been isolated on *S. elaeagnifolium* in southern Arizona for at least 300 generations.

The Alberta population has been maintained as a breeding colony in the laboratory for several years. To avoid genetic drift and/or artificial selection, it is supplemented each year with wild insects collected from potato gardens in the Edmonton area. Potato is the

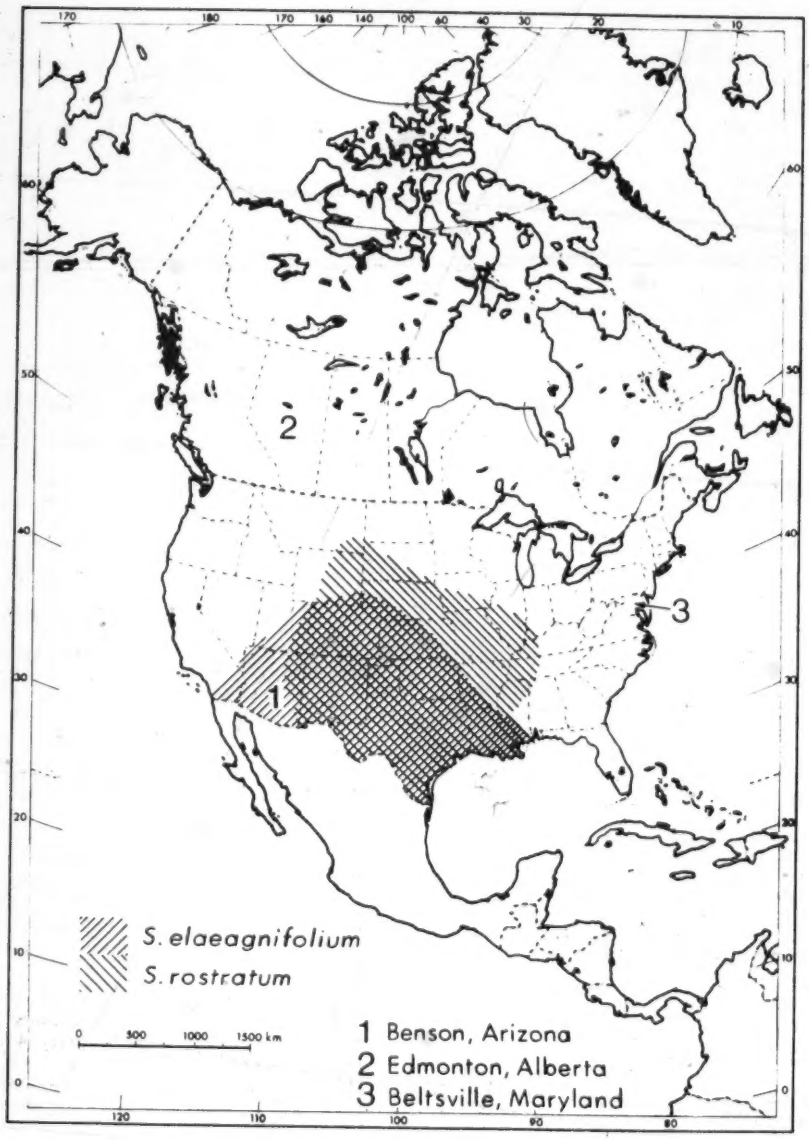

Figure 1.1. Origins of the three populations of *L. decemlineata* used in this study and estimated distributions of their two primary *Solanum* host plants in North America. (Sources: Whalen 1979, Rickett 1979).

primary host for *L. decemlineata* throughout western Canada; however beetles are occasionally reported feeding on both wild and domestic tomato. This population is a descendant from the original colonists on potato and has been largely isolated on potato for at least 150 generations.

An eastern population of Colorado potato beetles that feed on tomato (*Lycopersicon esculentum* Mill) was obtained from the Beltsville Agricultural Research Center in Beltsville, Maryland. Beetles from this area are collected from tomato fields and reared on the same plant in the laboratory. Although reports of *L. decemlineata* feeding on tomato date back to Tower (1906), the insect has only recently been elevated to "pest" status on this plant in areas of intense tomato cultivation in eastern North America. Other potential host plants in the region include potato and several species of wild *Solanum*.

Beetles were reared in active breeding colonies in the laboratory on their respective regional host plants as described in Appendix I. Procedures followed for the growth and maintenance of plants are given in Appendix II.

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II. Host plant discrimination and its evolution in the Colorado potato beetle *Leptinotarsa decemlineata*

Introduction

Host selection by phytophagous insects has traditionally been viewed as a sequential process involving the related phases of host finding and host recognition (Thorsteinson 1960, Miller and Strickler 1983). Due to the inherent problems associated with observing free-flying insects, analysis of long range orientation to a plant or its habitat has been somewhat neglected (but, see Ahmad 1983 for a recent review), with a resulting emphasis on more proximate interactions between insects and plants. The study of insect chemoreception (both taste and smell) has received special attention. It is with the aid of these senses that the "final decision" to accept a plant for feeding or oviposition is made.

In developing preliminary hypotheses concerning how insects identify their host plants, researchers drew from prevailing models in ethology and vertebrate neurobiology. The inital discovery that glucosinolates (mustard oil glycosides) in cruciferous plants acted as strong feeding stimulants for certain insects (Verschaffelt 1910) stimulated research into the effects of secondary plant compounds on insect behaviour. Summarizing the early literature, Fraenkel (1959) concluded that these compounds acted as a class of "token stimuli" (Tinbergen 1951) to insects. Evidence for the neurological correlate of token stimuli — "labelled lines"[1] — came later when Schoonhoven (1967) described a chemoreceptive cell in *Pieris brassicae* sensitive to glucosinolates.

Failure to extend the token stimulus concept far beyond the Cruciferae and their associated insects (see Dethier 1982) may have led, in part, to the hypothesis that secondary compounds defined host ranges by signalling to insects what *not* to eat (Jermy 1958, 1961). This idea was consistent with growing evidence that many secondary plant compounds (e.g. nicotine, strychnine, the pyrethroids) were toxic to insects. Although this hypothesis

---

[1]labelled lines are thought to be hard-wired neural channels which respond to single classes of stimuli and result in specific behaviours.

proposed a different mode of action for secondary compounds, the concept of single factor regulation of plant choice remained, and numerous experiments followed that claimed to support Jermy's view (see Chapter 3, for a critique). Similarly, more detailed studies designed to investigate the control of food intake in insects (e.g., Hamamura et al. 1962) were modelled in simple linear, stimulus-response terms, as attempts were made to isolate from plants the appropriate "sign stimuli" (e.g., biting factors, swallowing factors) that were presumed to release each step in a behavioural sequence. Together, these approaches helped propagate a somewhat static and simplistic view of behaviour in phytophagous insects.

More recently, ideas concerning the control of host choice by insects have shifted to include more dynamic concepts involving the integration of patterned sensory input and the perception of total plant "Gestalts" (Dethier 1970, 1973, 1976, 1982). These ideas grew from the discovery that insect chemosensory neurons, like their vertebrate counterparts, are generally not highly specific, but instead have broad and overlapping response spectra (Schneider 1969, Dethier 1973, 1974, 1977; Blaney 1975; van Drongelen 1978 et al.). The current view is that sensory quality in these systems is coded not by individual labelled lines (although these may be a factor), but by large populations of interacting neurones which produce unique "across-fibre" (Pfaffman 1941, Erickson 1967) patterns of response.

Increasing understanding of how these complex systems function in discrimination among plants will require new directions in research. At the neurophysiological level, Dethier and Crnjar (1982) have attempted to determine how plants of varying quality are identified by insects through a detailed analysis of the patterns of sensory input produced across gustatory neurones exposed to plant saps. To date, no complementary approach has been taken at the behavioural level of analysis. Early ethological studies (Williams 1954, deWilde 1958, Zohren 1968) provided descriptions of preliminary behaviours (drumming plant with forlegs, palpating, nibbling, and so on) performed by phytophagous insects prior to feeding or oviposition. Later, when the functional significance of these behaviours in terms of plant sampling became apparent, more detailed analyses proved useful in exploring the effects of

glucosinolates on plant acceptance in aphids (Nault and Styer 1972) and oviposition in the cabbage fly *D. brassicae* (Nair and McEwen 1976). However, studies of this kind have focused only on those species in which the behaviours are regulated largely by single compounds.

In this paper, I examine the broader question of perception of overall plant quality through analysis of entire sequences of plant assessment and feeding in the Colorado potato beetle *Leptinotarsa decemlineata* (Say). This insect is an appropriate model organism to use, since both olfactory discrimination based on complex mixtures of stimuli (Visser and Ave 1978, Ma and Visser 1978), and absolute discrimination based on gustatory inhibition by alkaloids (Hsiao and Fraenkel 1968, Bongers 1970), have been implicated from previous experiments. My first objective in this study was to determine whether measurable variations in behaviour patterns could be used to indicate how beetles discriminate among closely related plant species. My second objective was to use the behavioural data to gain insight into the role that underlying neural mechanisms play in the evolution of host choice by comparing three recently evolved geographic populations of insects which use different host plants (see Chapter 1, Study organisms).

Materials and Methods

Insects used in this experiment were collected in the field at three different locations (see Chapter 1, Figure 1.1) and reared in the laboratory on their respective regional host plants. Twelve beetles from each geographic population were randomly assigned to each of four plant groups, *Solanum tuberosum* ("Norland" variety), *Solanum rostratum*, *Solanum elaeagnifolium*, and *Lycopersicon esculentum* ("Earliana" variety). These plants represent the three regional hosts and presumed ancestral host of the species (see Chapter 1, Study organisms). All beetles were tested as naive (no feeding experience) adults in their first day after emergence.

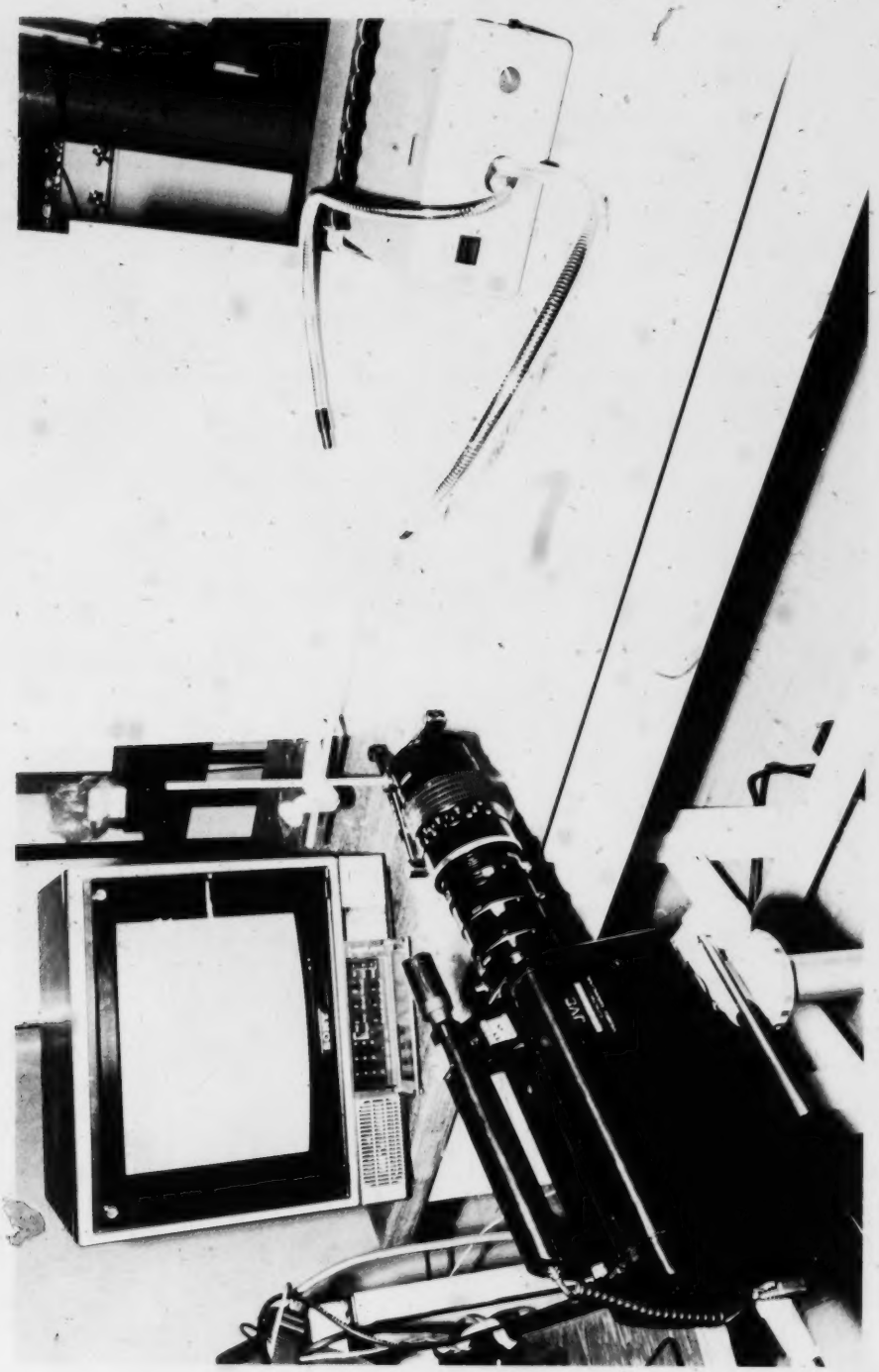
Each insect was tested individually with a single, separate leaf using a simple apparatus designed to allow videotape monitoring of behavioural events associated with feeding (see Figure 2.1). This apparatus consisted of a teflon rod held horizontally on a stand and split lengthways at one end to hold a leaf, whose petiole was placed in water. Beetles were transfered to the apparatus by inducing them to crawl up a second teflon rod which was then placed against the test rod as the insect walked. Leaf outlines were traced before and after feeding to provide a permanent record of leaf consumption.

Insect behaviour was recorded using a 0.5 inch format videotape system consisting of a JVC color video camera (model #HR7300U), a Panasonic cassette recorder (model #NV-8420), and a Sony CVM-1270 color monitor. Equipped with a C-mount, the camera could be adapted for use with standard photographic lenses. I used a Micro-Nikkor 105mm lens and two M-rings to achieve the desired level of magnification. Light was directed on the leaf using a Volpi Intralux 5000 fibre optic system. Unlike film units, video cameras require only moderate levels of lighting, well within the tolerance range of the insects, to achieve optimum resolution. Behaviour was videotaped for a minimum of three minutes from the time beetles first contacted the plant. This allowed repeated observations of the initial sampling behaviours on the leaf. Events that ocurred after the three minute videotape period were observed on the monitor and noted. Cumulative-time stopwatches were used to time all events.

Beetles were allowed free access to a leaf until three minutes without feeding elapsed. This period was previously determined as a minimum inter-meal interval time for this insect. Therefore, unlike long term feeding studies, this experiment focused on sensory aspects of plant discrimination by minimizing post ingestive effects on feeding.

In a preliminary study of beetles feeding on potato leaves in petri dishes, first meal size of females (Mean=27 mm^2, SD=12.0, n=10) and males (Mean=20 mm^2, SD=9.0, n=9) did not differ significantly (Kruskal-Wallis test, H=1.74, 1 d.f., P>0.10). Therefore, because supplies of new adults were limited, both sexes were used and assigned to

Figure 2.1. Equipment setup used for videotaping and monitoring insect behaviour during feeding experiments.


JVC

groups without knowledge of sex (sexes are indistinguishable unless examined with a microscope).

Plants used for both experiments and insect culture food were grown in a greenhouse under standard conditions (see Appendix II). *S. tuberosum* was grown from tubers, while *L. esculentum*, *S. rostratum*, and *S. elaeagnifolium* were all grown from seed. To minimize effects due to inter-plant variability, leaves from at least six plants of each species were used in the feeding tests for each population of beetles. All leaves were taken from healthy plants and only leaves from the second or third node below the apical tip (21 days to 42 days old) were used.

Leaf consumption was measured using a Licor Area Meter, model #3100 (accuracy=1mm^2). Since accurate estimates of leaf thickness could not be obtained, leaf areas consumed were corrected for differences in thickness using their weight:area ratios. Twenty-five leaf discs of equal size were cut from each of the four plant species and weighed. Leaf discs of *S. tuberosum* (mean=10.6 mg, SD=0.30), *S. rostratum* (mean=10.5 mg, SD=0.20), and *S. elaeagnifolium* (mean=10.4 mg, SD=0.20) were approximately equal in weight. Leaf discs of *L. esculentum* (mean=6.9 mg, SD=0.21) were on average 34% lighter than the three *Solanum* species. Therefore, each value for *L. esculentum* was reduced by 34% and values for the three other species were left unchanged.

Data Handling

Because of small sample sizes, certain estimates of populations means were strongly biased by extreme scores. Since there was no *a priori* reason for discarding extreme values, I opted to *winsorize* them (see Sokal and Rohlf 1981). In this procedure, the extreme score is assigned the value of its nearest neighbour in rank. Potential outliers in each sample were first tested for statistical significance by the Dixon test. Outlier values, samples from which they came, Dixon test results, and assigned values are as follows:

(1)Alberta, *S. elaeagnifolium*/gustatory sample: score=20s (Dixon test r=.50, n=10,

$P<.05$); assigned value=11s.

(2)Arizona, *S. rostratum*/explore: score=32s (Dixon test $r=.79$, $n=12$, $P<.01$); assigned value=9.5s.

(3)Arizona, *L. esculentum*/explore: score=25s (Dixon test $r=.80$, $n=8$, $P<.01$); assigned value=12.5

All statistics were calculated using BMDP statistical software (Dixon 1983).

Results

Behaviour protocols

Sequences of adult *L. decemlineata* behaviour on primary host plants generally followed a stereotyped and ordered pattern of sampling, feeding, grooming and rest (Figure 2.2). With the exception of the "small bite phase", which is sometimes absent and tends to be indistinguishable from "sweep-feeding", the behavioural categories used could be reliably timed using clear beginning and end-points. For illustrations of categories A through D below, see Figure 2.3.

A. *Explore*: Duration on the leaf surface beginning with first physical contact with the leaf and ending with transition to *gustatory sampling*. Active movements include walking, palpating (repetitive contacts by the maxillary palpi on the leaf surface) and antennal waving. Sensory input, in addition to that from antennae, maxillary palps and labial palps, may also be received from sensilla situated on the tarsi.

B. *Gustatory sample:* Before feeding, exploring the leaf surface was followed by a period of "squeezing" the plant with repetitive movements of the mandibles. This typically occurred on the leaf edge, but beetles occasionally sampled leaf veins in a similar manner. On acceptable plants, this piercing action of the mandibles released visible droplets of plant fluid,

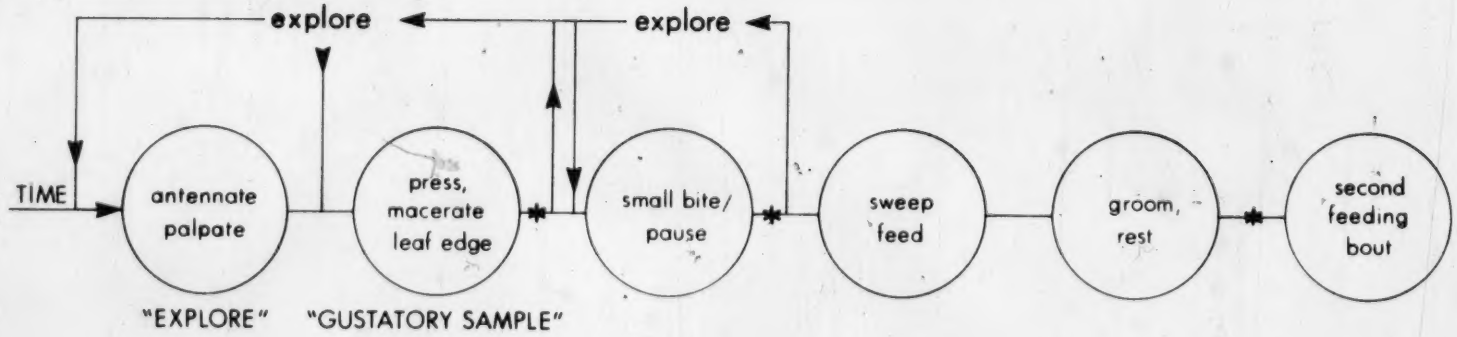
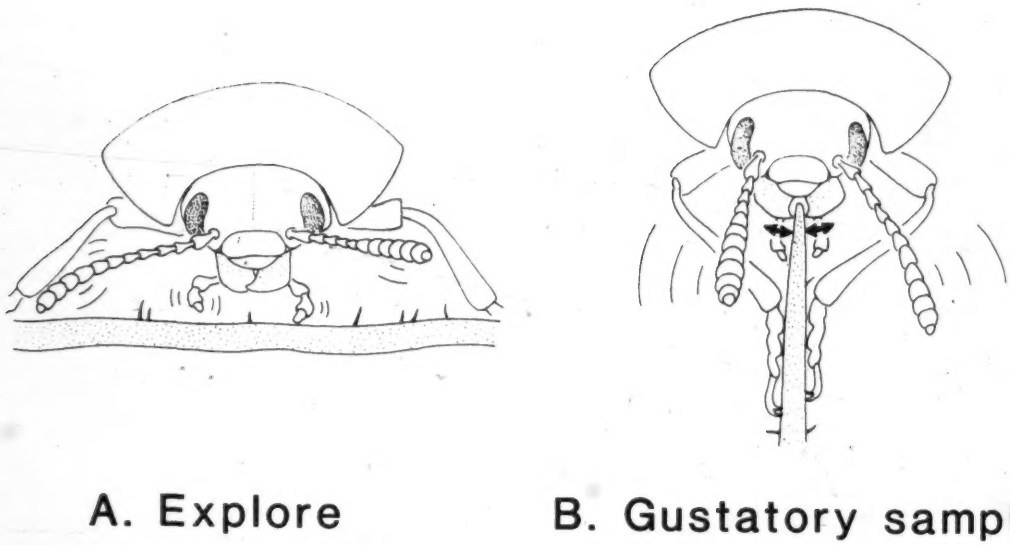


Figure 2.2. Plant sampling and feeding behaviour by *L. decemlineata* adults on a primary host. Ethogram illustrating a typical behavioural sequence from the time of initial plant contact through to the termination of first meal feeding.



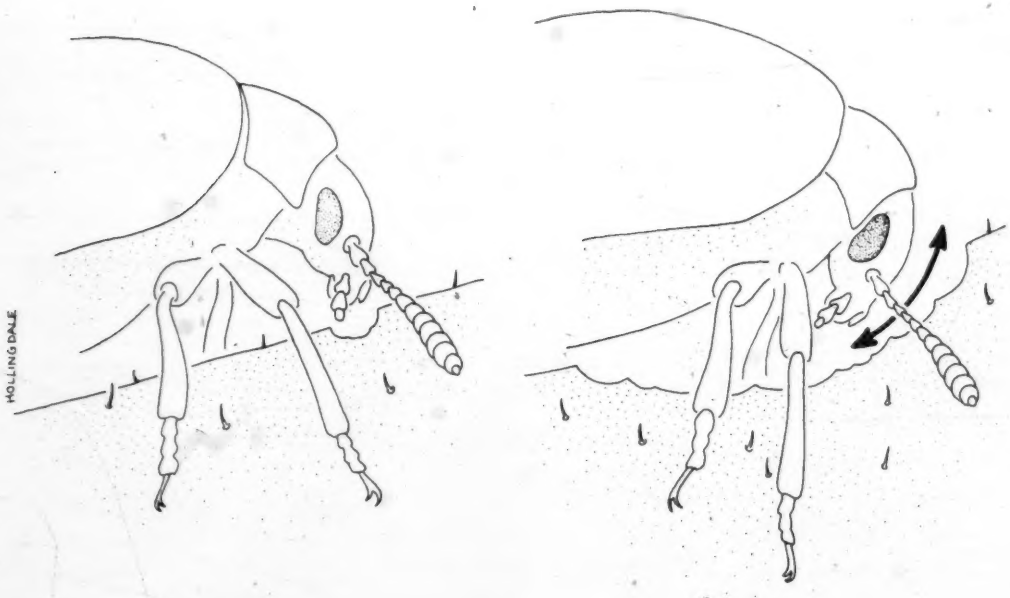


Figure 2.3. Four component behaviours in the transition from plant assessment to feeding by *L. decemlineata* adults.

bringing gustatory sensilla on the insect's mouthparts into direct contact with leaf sap (thus the name gustatory sampling). On plants that were not eaten, however, action of the mandibles was often less vigorous, and gentle "pressing" of the leaf edge did not result in apparent breaks in leaf tissue. This behaviour is thus graded and depends on plant quality, but no attempt was made to quantitatively distinguish "pressing" from "macerating".

While I use the term gustatory sampling for convenience, it is not meant to imply that the only function of the behaviour is to stimulate gustatory sensilla on the mouthparts. As indicated in Figure 2.3(B) the antennae are actively drawn towards the sampling site during this phase. Thus, the movements of the mandibles may also serve to enhance the release of plant volatiles. The duration of the first gustatory sampling bout and the frequency of bouts during the test period were recorded.

C. *Small bite*: A bite was noted when a visible fragment of leaf was taken into the oral cavity. In most cases, biting involved distinct movements (bite through leaf with mandibles, lift head), and could therefore be easily distinguished from gustatory sampling. Beetles normally (but not always) took one or two small bites and paused while chewing the leaf fragments.

D. *Sweep feed:* Rapid feeding characterized by repetitive bites as the head is moved in a rhythmic, sweeping fashion. While small biting and sweep feeding are distinguished here to give the reader a "feel" for the gradual progression to full feeding, the two behaviours could not always be clearly separated. Subsequent use of the term "feeding" will therefore refer to both behaviours. Duration of feeding, measured from the time of first bite, was used to calculate feeding rate. The number of different feeding sites on the leaf was also recorded.

Feeding bouts were normally terminated by *grooming*, or *rest*, and occasionally by further examination of the plant surface. Rest was defined as a period of thirty or more seconds of

motionlessness.

Comparisons among plants: within insect populations

The relatively stereotyped sequences of *L. decemlineata* behaviour broke down on plants less preferred for feeding (for example, see Figure 2.4). On preferred host plants (plants eaten the most) beetles usually sampled and fed at a single site on the leaf before ending the feeding bout with a long (greater than 3 minutes) period of grooming. On plants that were eaten less however, fewer insects proceeded directly through the sequence, and many re-initiated sampling and feeding at numerous sites on the leaf before stopping to rest or groom.

In addition to these pattern differences, there were consistent trends in several of the single variables measured in relation to plant species preference. In Tables 2.1-2.3 behavioural data are arranged within each population by plant species ranked[2] in terms of consumption. On average, reduced feeding on a plant species was associated with increased exploration time prior to gustatory sampling and a longer gustatory sampling period for those insects that proceeded to feed. There was also an increase in the number of sampling and feeding sites on lower ranked plant species during a single feeding bout. However, when acceptance of a species by beetles from a population approached nil (e.g., *L. esculentum* for Arizona and Alberta groups), rejection of individual plants often occured after just one gustatory sampling event. Note that these trends were consistent although plant species were ranked differently by the insects within each population.

Not all differences in feeding and sampling behaviour were statistically significant. Where consumption differences among plant species were large, associated sampling behaviours also tended to vary significantly (e.g., the Alberta population, Table 2.1). However, variability in sampling behaviour was not statistically significant within the Maryland population, where differences in consumption among plant species was relatively

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[2]a plant's rank refers to consumption on that plant relative to the other species tested

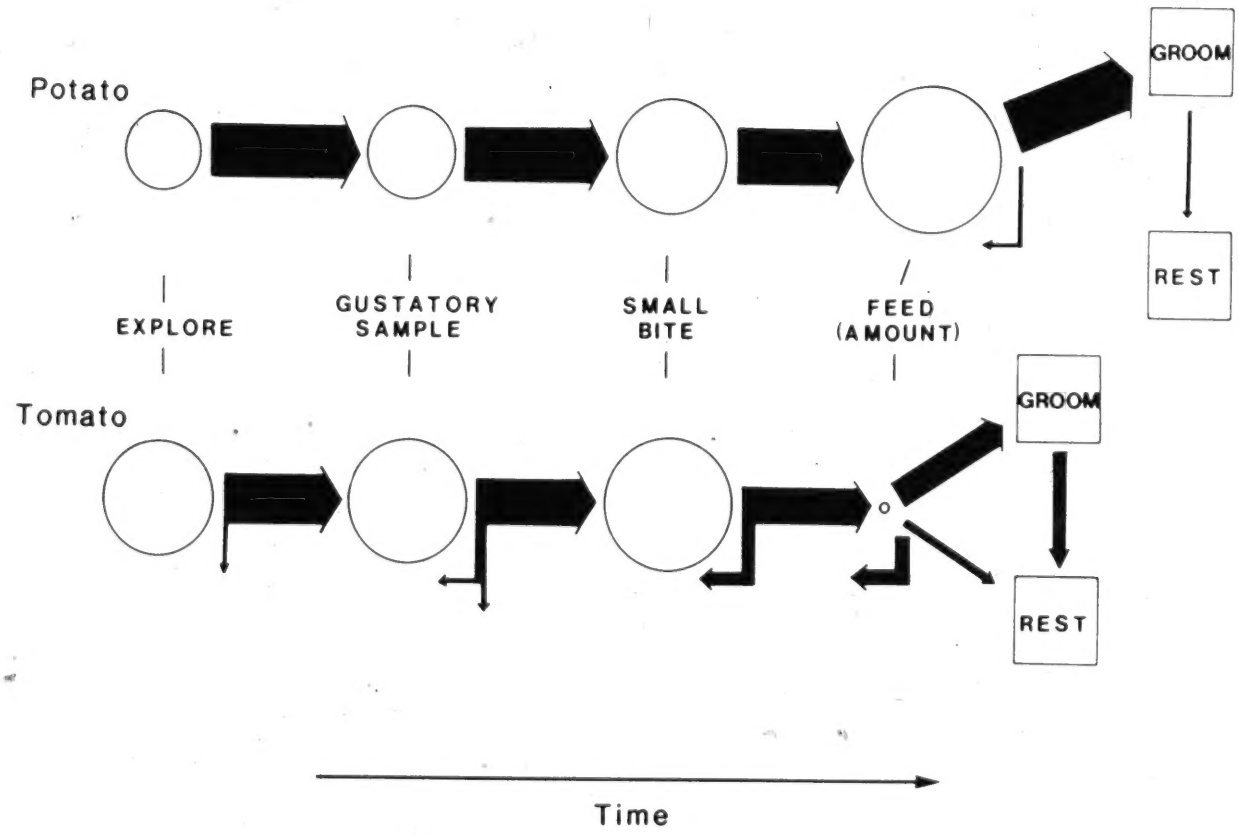

Figure 2.4. Kinematic graph depicting variable behaviour patterns by beetles from Alberta on their preferred regional host (potato) and a marginal host plant (tomato). Observations are from 12 beetles per plant. Areas of the circles are proportional to the time spent in each behaviour except Feed which is proportional to the amount of leaf consumed. Behaviours that typically terminated the first meal (in squares) were recorded for three minutes after feeding stopped. Width of the arrows is proportional to the number of animals in transition. Arrows pointing back refer to beetles which repeated an earlier behaviour in the sequence; arrrows pointing down to those that walked off the plant and did not return during the test period.

Table 2.1. First meal feeding and associated sampling behaviours by beetles from Alberta tested on four solanaceous plants ranked by consumption (Amount). Values given are means±standard errors or medians, range.

	FEEDING				SAMPLING		
Plant	#Feed/n	Amount (mm^2)	Rate (mm^2/min)	#Sites	Exp[1]s	Prefeed GS[2](s)	#GS Sites
S.rostratum	12/12	82±15	3.3±.33	1.0,1-3	2.4±0.41	3.1±0.55	1.0,1-2
S.tuberosum	12/12	72±11	9.0±.98	1.0,1-1	3.3±0.33	3.7±0.43	1.0,1-1
S.elaeagnifolium	10/12	43±10	3.1±.70	2.0,1-6	3.4±0.50	5.5±1.20	2.5,1-9
L.esculentum	10/12	5±2	--	2.0,1-3	4.9±0.95	5.5±0.84	1.5,0-4
	G=5.91[3] 3df NS	F=8.26*** 3,18df	W=15.1*** 2,13df	G=8.20[4]* 3df	F=2.94* 3,43df	F=2.70[3] 3,40df P=0.06	G=16.6[4]*** 3df
Levene's test (log transformed)		F=2.15 3,39df NS	F=3.66* 2,27df		F=1.31 3,43df NS	F=2.02 3,40df NS	

[1]Exp=duration *exploring* leaf surface prior to first *GS*.
[2]GS=duration *gustatory sampling* leaf prior to biting (note:feeders only included).
[3]calculated by grouping into feeders and non-feeders.
[4]calculated by grouping into categories of 1, and >1.
* P<.05, ** P<.01, *** P<.001

Table 2.2. First meal feeding and associated sampling behaviours by beetles from Arizona tested on four solanaceous plants ranked by consumption (Amount). Values given are means±standard errors or medians, range.

	FEEDING				SAMPLING[1]		
Plant	#Feed/n	Amount (mm^2)	Rate (mm^2/min)	#Sites	Exp(s)	Prefeed GS(s)	#GS Sites
S.elaeagnifolium	12/12	44±5.6	3.6±.74	1.0,1-2	2.4±0.22	3.0±0.31	1.0,1-2
S.rostratum	12/12	33±5.4	3.4±.43	1.0,1-3	4.6±0.82	3.3±0.50	1.5,1-3
S.tuberosum	2/12	12±6.0[2]	---	2.5,1-4[2]	14.3±3.78	12.4±1.10[2]	6.5,2-11
L.esculentum	1/12	---	---	---	6.3±1.5[3]	---	1.0,0-4
	G=48.1*** 3df				W=6.77** 3,16df		G=22.7*** 3df
Levene's test (log transformed)					F=10.57*** 3,39df		

[1]Abbreviations as in Table 2.1.
[2]n=2 feeders only.
[3]n=8, four insects did not proceed to *GS* stage.
**P<.01, *** P<.001

Table 2.3. First meal feeding and associated sampling behaviours by beetles from Maryland tested on four solanaceous plants ranked by consumption (Amount). Values given are means±standard errors or medians, range.

	FEEDING				SAMPLING[1]		
Plant	#Feed/n	Amount (mm^2)	Rate (mm^2/min)	#Sites	Exp(s)	Prefeed GS(s)	#GS Sites
S.tuberosum	12/12	45±9.7	4.1±.54	1.0,1-6	3.4±.52	2.5±.38	1.0,1-2
S.elaeagnifolium	11/12	41±7.1	2.1±.28	1.0,1-3	3.5±.47	2.8±.46	1.0,1-2
S.rostratum	12/12	28±4.0	2.1±.22	1.0,1-3	3.9±.65	3.2±.30	1.0,1-3
L.esculentum	8/12	21±5.9	3.1±.74	1.5,1-4	4.9±1.34[2]	3.7±.33	1.5,1-7
	G=9.90* 3df	W=2.44 3,20df NS	F=6.36** 3,34df	G=2.87 3df NS	F=0.70 3,42df NS	F=1.76 3,39df NS	G=0.64 3df NS
Levene's test (log transformed)		F=5.78** 3,39df	F=0.88 3,34df NS		F=0.41 3,42df NS	F=1.66 3,39df NS	

[1]Abbreviations as in Table 2.1.
[2]n=10, two beetles did not proceed to *GS* during test.
* P<.05, ** P<.01

small (Table 2.3).

Because most beetles from Arizona did not feed on *S. tuberosum* or *L. esculentum*, comparisons of measures related to feeding could not be made across all plant species within this population. Consumption of *S. elaeagnifolium* and *S. rostratum* by beetles from Arizona ($F=1.71$, d.f.$=1,22$, $P=0.20$), and duration of pre-feed gustatory sampling on these two plants ($F=0.19$, d.f.$=1,21$, $P=0.67$), were not significantly different.

Variability among beetles for acceptance of marginal plants also appeared to affect duration of the first gustatory sampling bout. On low ranked species at least, there is an indication that non-feeders reject plants after relatively short gustatory sampling bouts, while feeders tend to gustatory sample for a longer period before biting (Table 2.4). For this reason, only feeders are compared in Tables 2.1-2.3 with respect to the duration of the first gustatory sampling event. Non-feeders on the same plant also tended to explore longer than feeders, but the breakdown of animals into the two groups did not provide sufficient sample sizes to test this observation statistically.

To test for homogeneity of within group variances, I transformed the data to logarithms and applied Levene's test (Tables 2.1-2.3). This procedure is equivalent to testing for differences in coefficients of variation by correcting for relative changes in variance that may accompany samples with different sized means (Sokal and Braumann 1981). Where there was significant heterogeneity of within group variances, I used Welch's one-way analysis of variance test statistic W to test for differences in sample means. Welch's test statistic is recommended as a robust alternative to the ANOVA F statistic if sample variances are not homogeneous (Brown and Forsythe 1974).

Correlations between any single pair of variables within beetle populations were generally weak. The following values, taken from the Maryland population, are typical: feeding amount and exploration time ($r=-0.19$, d.f.$=41$, $P>0.10$); feeding amount and duration of gustatory sampling ($r=-0.31$, d.f.$=41$, $P<0.05$); explore time and duration of gustatory sampling ($r=0.12$, d.f.$=41$, $P>0.10$). These values varied among populations,

Table 2.4. Comparison of initial sampling behaviours by feeders and non-feeders on the same plant species. Values are means±standard errors and (n).

	EXPLORE		GUSTATORY SAMPLE	
Population/Plant[1]	Feeders	Non-feeders	Feeders	Non-feeders
Arizona/S.tuberosum	3.4±1.65 (2)	16.7±4.23 (9)	12.4±1.10 (2)	6.9±1.59 (10)
Arizona/L.esculentum	1.0±---- (1)	7.0±1.54 (7)	8.4±----- (1)	3.9±1.35 (7)
Alberta/L.esculentum	4.4±0.90 (10)	9.8±----- (1)	5.5±0.84 (10)	3.9±---- (1)
Maryland/L.esculentum	3.2±0.60 (8)	11.4±4.05 (2)	3.7±0.33 (8)	1.7±0.70 (2)
Maryland/S.eleaegnifolium	3.4±0.51 (11)	4.6±---- (1)	2.8±0.46 (11)	5.9±---- (1)
Alberta/S.eleaegnifolium	3.4±0.61 (10)	3.7±0.10 (2)	6.0±1.50 (10)	9.9±6.20 (2)

[1]on a population basis, first four plants listed are ranked low; the last two moderate.

and no single variable was a good predictor of plant preference.

Differences in feeding rate were not related to host preference, but reflected the physical characteristics of the plants. Both *S. rostratum* and *S. elaeagnifolium* have leaf surfaces covered by dense mats of trichomes which visibly interfered with feeding. As a result, feeding rates on these two plants were consistently lower than the other species tested. This physical interference did not seem to affect the size of the first meal however, since both plants were ranked highly in all populations.

Behaviours terminating feeding sequences generally did not vary in relation to host quality. Regardless of meal size, beetles normally ended feeding by grooming for several minutes and the sporadic occurrence of grooming during feeding bouts was not systematically related to plant type. Beetles from Alberta feeding on tomato were the one possible exception to this rule. Most of those insects initiated feeding, but stopped quickly and groomed for only a short period (if at all) before resting, perhaps indicating the operation of a post-ingestive mechanism affecting food intake.

Comparisons between and across insect populations

Consistent trends within beetle populations between sampling behaviour and plant consumption suggested that combining population data to further investigate the nature of these relationships might be appropriate. However, first meal sizes (and feeding rates) of the Alberta population were considerably higher than those from Maryland and Arizona (see Tables 2.1-2.3). Also, I could not equalize the within group variance of all samples with transformations.

As an option to performing parametric regression analysis on the entire data set, mean sampling and feeding data were pooled from the Arizona and Maryland populations (which did not appear to be inherently different in terms of feeding behaviour) and analysed using Kendall's rank correlation test (suggested as a nonparametric test of trends on means by Sokal and Rohlf 1981). Both explore time and gustatory sampling were significantly

negatively correlated with plant consumption using this analysis (Figs. 2.5 and 2.6). Data for the Alberta population are also provided in Figures 2.5-2.6 but correlation coefficients were not computed because at least five data points are needed for the analysis.

Two individual comparisons were of interest with respect to adaptation to novel hosts. Beetles from Alberta that have colonized potato and are descendant from a wild *Solanum* feeding stock (like the Arizona population), spent a shorter time in both kinds of pre-feed sampling behaviour on potato than beetles from Arizona. However, the Maryland population, which is descendant from a potato feeding stock (like beetles from Alberta) and is in an earlier phase of colonizing *L. esculentum*, explore on the new plant for an equal length of time as beetles from Alberta, but gustatory sample somewhat less (although not significantly less, $t=1.38$, 16 df, $P=.09$; one-tailed test on log transformed data). This raises the possibility that adaptation to *L. esculentum* may first involve changes in sensitivity at the gustatory level.

Discussion

Mechanisms of Discrimination

My objective in this study was to investigate mechanisms of host plant discrimination in *L. decemlineata* adults by using a behavioural analysis. The results indicate that beetles are capable of graded, sensory-based discrimination of plants, over a range of species normally used by the insects in nature. This conclusion is based on the consistent inverse relationship between pre-ingestive sampling behaviour and food consumption across plant species. Previous electrophysiological and behavioural studies with *L. decemlineata* adults have shown that longer-range orientation to a host is dependent on precise ratios of green leaf volatiles which are coded by olfactory receptors on the antennae (Visser and Ave 1978, Ma and Visser 1978). In this study, beetles were apparently capable of recognizing different plants on the basis of olfactory and contact chemoreceptive input alone, without reference to

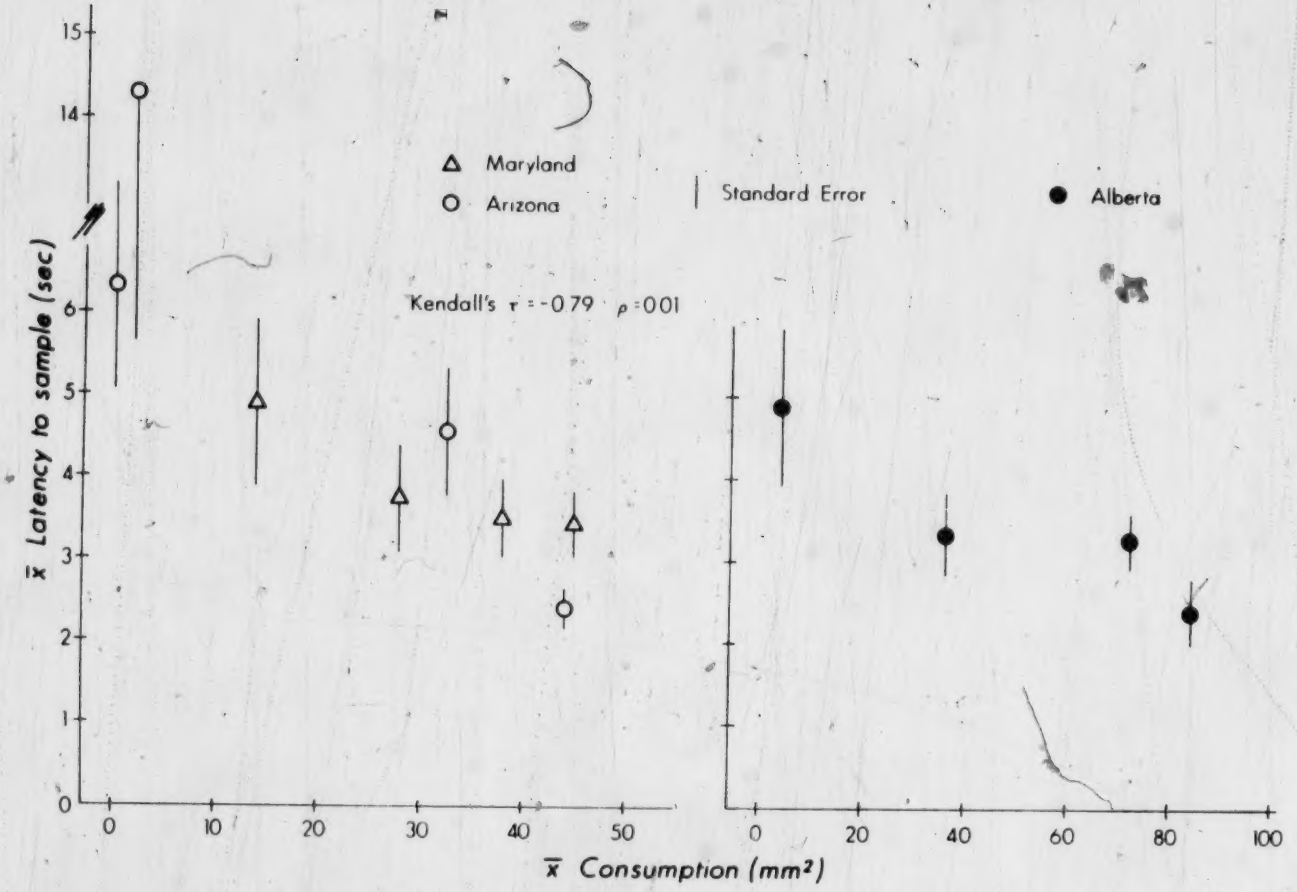

Figure 2.5. Mean latency to gustatory sample (exploration time) in relation to average food consumption on four species of plants for three beetle populations. Error bars for consumption omitted for clarity. N = 12 per plant.

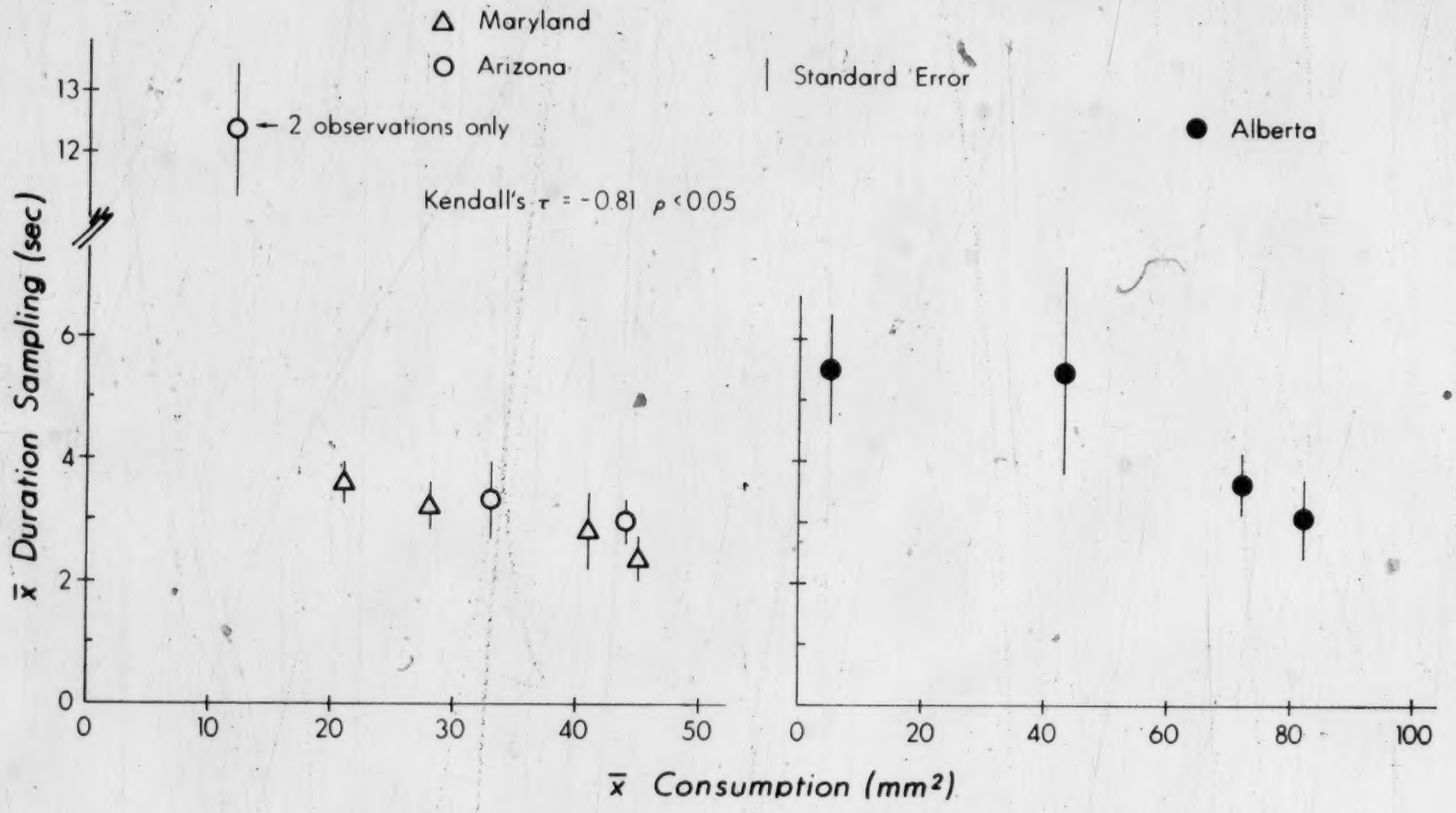

Figure 2.6. Relationship between mean gustatory sampling time and average consumption on four species of plants for three beetle populations. Error bars for consumption omitted for clarity. N = 12 per plant.

internal plant saps. This finding plus the observation that the antennae are actively drawn towards the leaf during gustatory sampling suggest a dominant role for olfaction after the beetles contact the plant as well.

Although two categories of sampling behaviours (explore and gustatory sample) have been identified in this study, there is no reason to presume they involve separate underlying mechanisms. Input from mouthpart receptors in phytophagous insects typically converge with antennal input in the brain after only one synapse at the suboesophageal ganglion (Chapman 1982). And, as noted previously, antennal input is probably intensified by the same behaviour that initially exposes gustatory neurones to plant saps. Transition from "exploring" to "gustatory sampling" may involve a serial integration of sensory information to a single CNS site.

The additional sampling of plant fluids does, however, provide the opportunity for secondary plant compounds to act as feeding deterrents, perhaps by interfering with "normal" sensory messages (Schoonhoven 1982, Mitchell and Sutcliffe 1984). If this were the case, time spent by beetles in gustatory sampling might be expected to vary independently of exploration time on plant species less preferred for feeding. In this study, however, increased gustatory sampling time by feeders on low ranked species was consistently associated with longer initial exploration times (Tables 2.1-2.3). Also, non-feeders that reject individual plants after short gustatory sampling bouts tend to explore on the leaf surface first for much longer periods than feeders on the same plants (Table 2.4). These observations suggest that beetles discriminate among plants primarily on the basis of mixtures of plant volatiles and surface compounds, and not on the basis of inhibitory compounds (i.e., glycoalkaloids) in the plant saps.

A lower mean (but statistically insignificant) gustatory sampling period by Maryland beetles on *L. esculentum* as compared to Alberta beetles is the one notable exception to this pattern, and raises the possibility that adaptation by insects in Maryland to this plant involves a decreased sensitivity to the tomato alkaloid tomatine.

Evolution of host choice

Evolutionary changes in host choice by phytophages have attracted considerable interest because of the potential for host-specific populations of insects (biotypes or host races) to develop into new species (Dethier 1954, 1970; Futuyma 1983, White 1978). In terms of underlying neurological mechanisms, colonization of new host plants by oligophagous insects may be associated with any combination of changes at the primary receptor level or in the CNS, such that new combinations of plant compounds are either detected, or interpreted differently, by the sensory system (Schoonhoven 1977).

To date, few attempts have been made to address this question. Best studied are a group of related moth species in the genus *Yponomeuta* which have evolved different host preferences (Herrebout et al. 1976, Schoonhoven et al. 1977, van Drongelen 1980, van Drongelen and van Loon 1980, review by Futuyma 1983). Correlations have been shown between the moths' electrophysiological and behavioural responses to certain host chemicals (secondary compounds and sugars) and patterns of host use in the field. While such functional changes in the sensory system will develop at some point during the speciation process (individual species do have unique response characteristics), they are unlikely to be factors which mediate initial rapid colonization of new plants.

An alternative scenario is suggested by the results of this study on more recently evolved biotypes of *L. decemlineata*. It is clear from the large differences in behaviour on marginal host plants, that considerable variability exists within populations in terms of host acceptability. Interestingly, individual insects that do feed on marginal plants have a tendency to sample these plants less prior to feeding (Table 2.4) i.e., they *perceive* the plants as more acceptable. As a new plant is colonized, it may be that these more catholic feeders are selected for, bringing about a temporary pool of generalist feeders which, after some longer period of association with the new plant, may undergo "fine-tuning" of the sensory system resulting in behavioural specificity for the new host.

Thus, the three geographic populations of *L. decemlineata* studied from North America have not totally lost their behavioural preference for the ancestral host, *S. rostratum*, despite being isolated[3] from the plant for up to several hundred generations (see Chapter 1). Figure 4.1 (Chapter 4) illustrates how populations in Alberta and Maryland have increasingly become generalists as new plants are added to the insects' repertoire of acceptable species during each colonization event. Other insect species that have been studied during the preliminary stages of plant colonization have also undergone expansion of feeding or oviposition preference, not direct behavioural shifts involving loss of preference for the old host (see Chapter 4).

Although not statistically significant, reduced feeding on *S. rostratum* by Arizona and Maryland beetles is associated with a slight increase in pre-ingestive sampling behaviour. This implies that functional changes in the chemosensory system are emerging within these populations that eventually may result in loss of preference for the ancestral host.

In summary, *L. decemlineata* beetles show graded preference for potential host plant species. A systematic increase in pre-ingestive sampling behaviour on less preferred species suggests a complex of stimuli is involved in plant discrimination. Olfactory input from plant volatiles and surface waxes may be especially important in this process. Colonization of novel host plants by regional populations of beetles has involved expansion of feeding preference. However, the fact that Maryland beetles rank their regional host (tomato) last of the four species tested, suggests adaptation to new plants may evolve relatively slowly, perhaps due to the complexity of mechanisms controlling host choice behaviour. This possibility is discussed in Chapter four.

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[3]Gene flow between the southern U.S. where *S. rostratum* occurs and the three regions sampled in this study is expected to be low. Large, dispersing populations of this insect develop only on agricultural crops.

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III. Effects of solanaceous alkaloids on plant sampling and feeding behaviour by adult Colorado potato beetles

Introduction

Secondary plant compounds are a diverse group of chemicals common to all vascular plants. Individual plant families, genera, or even species may be characterized by their own unique complement of these compounds (Robinson 1980, Harborne and Turner 1984). For example, Cruciferae contain mustard oil glycosides while the Solanaceae are known for their various alkaloids. Typically bitter tasting to humans, many secondary compounds are toxic or interfere with digestion in herbivores (see Rosenthal and Janzen 1979) and many act as feeding deterrents. However, others (especially the mustard oil glycosides) are known feeding stimulants for certain oligophagous insects (see Dethier 1982).

Current theory states that host specificity in plant feeding insects (and ultimately patterns of insect-plant evolution) is based on the defensive properties of secondary compounds which limit the number of plant species a given insect can use (Fraenkel 1959, Ehrlich and Raven 1964, Feeny 1975). Some authors have envisaged an evolutionary "arms-race", with plants adapting to herbivore selection pressure by developing new forms of toxic compounds, and insects counter-adapting physiologically through improved detoxification abilities, or behaviourally by evolving sensory recognition of the new compounds to aid them in identifying plants.

More recently, active roles in plant metabolism have been discovered for most secondary compounds investigated, and their function in terms of plant defense has been questioned (Robinson 1974, Seigler and Price 1976, Jones 1979). In addition, a new consensus is developing (following Jermy 1976) that insects have rarely coevolved with plants in a reciprocal gene-for-gene sense, but rather have adapted to existing plant chemistry which itself has evolved (and is evolving) in response to a multitude of selection pressures including non-insect herbivores and invading microorganisms (see Futuyma 1983). Nevertheless, there

is ample evidence that secondary compounds are a factor, if not always the dominant one, in shaping patterns of host plant use by phytophagous insects.

Especially interesting in terms of insect behaviour are those plant chemicals that may act directly on the sensory system. Research in this area was stimulated by the discovery that certain mustard-oil glycosides in the Cruciferae act as strong feeding attractants for the cabbage butterfly *Pieris rapae* (Verschaffelt 1910). Since then the role of secondary compounds (both as stimulants and deterrents) have been predominant in theories of host plant recognition by insects (Fraenkel 1959, Dethier 1970, 1982, Schoonhoven 1982).

Studies on the Colorado beetle, *L. decemlineata* (Say), have played an important part in the development of these ideas. Early studies with this insect concentrated on demonstrating negative effects on larval fitness from the ingestion of alkaloids derived from marginal hosts or non-host plants (see Lipke and Fraenkel 1961, Hsiao 1974 for summaries of the early literature). Also, experiments with *L. decemlineata* adults demonstrated some reduction in feeding on natural plants with the addition of the tomato alkaloid tomatine (Stürckow and Löw 1961, Bongers 1970). None of the these studies directly investigated the mode of action of the alkaloids, yet in many of these papers and the reviews which followed (for example, Harborne 1982, Schoonhoven 1982), it is either implied, or explicitly stated, that the compounds exert their effects directly on the chemosensory system.

In the only direct electrophysiological investigation of this possibility, Stürckow (1959) reported that tarsal sensilla in *L. decemlineata* adults were more sensitive to tomatine than solanine (a potato derived alkaloid). However, in a recent re-investigation of this topic, Mitchell and Harrison (1985) discovered the bursting type "responses" reported by Stürckow were actually injury effects which only developed after long periods of contact with pure compounds. There was no evidence of alkaloid receptors on the tarsi or the galea, nor were there different sensitivities, in terms of injury, across alkaloids tested (including tomatine and solanine).

While the above study suggests alkaloids may not function at the sensory level in *L. decemlineata*, it is not conclusive. There are epipharyngeal sensilla in this insect which are not accessable with current electrophysiological techniques. In addition, more subtle effects on the gustatory system, including inhibition of stimulant compounds at the receptor sites, are possible (Schoonhoven 1982, Mitchell and Sutcliffe 1984).

The experiments reported in this paper were designed to test the hypothesis that solanaceous alkaloids act as sensory inhibitors of feeding in *L. decemlineata* adults. Using videotape, direct observations were made on the beetles' behaviour during pre-ingestive sampling and first meal feeding on plants treated with alkaloids. It has previously been demonstrated that sampling of plant fluids ("gustatory sampling") by *L. decemlineata* beetles increases on plants less preferred for feeding (see Chapter 2). My objective in this study was to determine whether any reduction in feeding on alkaloid treated leaves was associated with similar changes in gustatory sampling behaviour; thereby demonstrating effects by the compounds at the sensory level.

Materials and Methods

Insects used in these experiments were collected in the field and reared in the laboratory on their respective regional host plants (see Chapter 1, Study organisms). All were first day, naive (no feeding experience) adults. In a preliminary study of Alberta beetles feeding on potato, first meal size of females (mean=27 mm^2 SD=12.0, n=10), and males (mean=20 mm^2, SD=9.0, n=9) did not differ significantly (Kruskall-Wallis test $H=1.74$, d.f.=1, $p>0.10$). Therefore, both males and females were used and assigned randomly (sexes are indistinguishable by eye) to treatment groups.

Plant sampling and feeding behaviour was recorded for each insect individually using an apparatus designed to allow videotape monitoring of behaviour (see Chapter 2, for details). Beetles were allowed free access to a leaf until three minutes without feeding elapsed. This period was previously determined by observation as a minimum inter-meal

interval time for this insect. Leaf area consumed was measured using a Licor Area Meter, Model 3100 (accuracy = 1mm²).

Plant leaves were treated chemically by passive transpiration. Leaf petioles were cut with a sharp razor blade, weighed, emersed in a narrow test tube containing the alkaloid solution, and sealed with Parafilm. Since alkaloids are non-volatile they accumulate in the leaf as transpiration occurs. From a knowledge of the percent water content of leaves, (Appendix III) and the concentration of the test solution, I was able to determine the approximate concentration of alkaloid accumulating in a leaf by weight loss of the solution in the test tube. Control leaves were placed in deionized water for a comparable length of time as treated leaves before being used. Throughout the course of the experiments, control and treatment leaves were interspersed randomly in time. All alkaloids used were obtained from Sigma Chemical Co.

To determine whether alkaloids were being distributed throughout the leaf and accumulating at the desired concentration, I conducted two tests. In the first, leaf petioles were placed in a water soluble flourescent dye and at periodic intervals photographed under UV light (which illuminated the dye in the leaf). After fifteen minutes, dye was visible throughout most of the leaf. Since treatment of plants with alkaloids took at least two hours, I am confident that test solutions were distributed evenly within the leaves.

In a second test, I attempted to directly measure the total glycoalkaloid content (TGA) of a group of alkaloid treated leaves. However, problems with the technique used (Fitzpatrick and Osman 1974, Fitzpatrick et al. 1978) when high concentrations of alkaloid were present (a precipitate formed in a separation phase) resulted in a TGA value of only 70% of that predicted. Since the time this test was carried out, Coxon (1984) has reviewed several studies that tested Fitzpatrick and Osman's technique and found recovery rates of standards highly variable and ranging from 46% to 78%. Given a minimum 22% loss, my analysis of treated leaves therefore accounted for at least (70+22)= 92% of the predicted alkaloid content.

In order to treat leaves to a desired TGA level, I also measured the resident glycoalkaloid levels in the three *Solanum* species used in these experiments (Appendix III). Levels of glycoalkaloid in *L. esculentum* were approximated by using values reported by Sinden et al. (1978).

Using the methods described above, three experiments were performed, each using a different insect population and plant combination.

Experiment 1

In this experiment I asked whether the initial rejection of *S. tuberosum* by Arizona beetles (see Chapter 2) was due in part to the presence of solanine in the leaves of this plant. A concentration of 1 mM solanine (at least twice the normal level found in potato) was infused into *S. elaeagnifolium* leaves and tested on seven beetles. An equal number were also tested on control leaves treated with distilled water. *S. elaeagnifolium* is the natural host of beetles in Arizona.

Experiment 2

This experiment served as a direct test of the hypothesis that reduced feeding on *L. esculentum* is caused by the alkaloid tomatine acting as a sensory deterrent. A total of twenty Maryland beetles were tested with either 2mM tomatine or water treated *L. esculentum* leaves. In this experiment the background level of positive stimulus input from the tomato leaves was presumably much lower than that from a primary host plant (Maryland beetles ranked *L. esculentum* fourth of four plants tested in Chapter 2). Since the control of food intake is thought to result from the balance of positive and negative stimulus input, any sensory based inhibition due to tomatine should have been readily detected.

Experiment 3

This study was designed to investigate the comparative effects of three alkaloids within a single beetle population. In addition to a control group, twelve Alberta beetles were individually videotaped on potato (*S. tuberosum*) leaves treated with a 2mM solution of solanine, tomatine, or atropine. Atropine is a tropane alkaloid with a different molecular structure than the steroidal alkaloids tomatine and solanine (Robinson 1980). Tropane alkaloids are common in several Solanaceae genera such as *Atropa* and *Datura* (Evans 1979) which are not hosts of *L. decemlineata*.

Results

Experiment 1

Sampling and feeding behaviour by Arizona beetles did not change with the addition of 1mM solanine to their regional host plant *S. elaeagnifolium* (Table 3.1). This alkaloid concentration represents twice that normally found in potato (which the insects largely reject) and several times the amount estimated in their regional host *S. elaeagnifolium*.

Experiment 2

Increasing the existing tomatine content in *L. esculentum* leaves by 2mM to yield an estimated TGA level of 2.5mM had no detectable effect on the behaviour of Maryland beetles (Table 3.2). Having one beetle reject a tomatine treated leaf was not unusual since one-third of the beetles tested on untreated leaves in an earlier experiment (Chapter 2) did the same. The slight reduction in feeding on tomatine treated leaves was not significant and there were no consistent trends in other variables measured.

Table 3.1. First meal feeding and associated sampling behaviours by beetles from Arizona tested on *S.elaeagnifolium* leaves treated with 1mM solanine or water (control). Values given are means±standard errors or medians, range. N=7 per treatment.

		FEEDING			SAMPLING		
Treatment	#Reject	Amount (mm^2)	Rate (mm^2/min)	#Sites	Exp(s)	GS(s)	#GS Sites
Control	0	21±3.8	2.4±.40	1.0,1-2	2.3±.63	3.3±.42	1.0,1-2
Solanine	0	24±2.4	2.8±.29	1.0,1-1	2.7±.27	2.6±.52	1.0,1-1
		F=0.54 df=1,12 NS	F=0.62 df=1,12 NS		F=0.17 df=1,12 NS	F=1.06 df=1,12 NS	

Exp=duration *exploring* on leaf surface prior to first GS
GS=duration of first *gustatory sampling* event prior to feeding.

Table 3.2. First meal feeding and associated sampling behaviours by beetles from Maryland tested on tomato leaves treated with 2mM tomatine or water (control). Values given are means±standard errors or medians, range. N=10 per treatment.

		FEEDING			SAMPLING[1]		
Treatment	#Reject	Amount (mm^2)	Rate (mm^2/min)	#Sites	Exp(s)	GS(s)	#GS Sites
Control	0	18±6.2	4.6±.73	1.0,1-2	1.8±.26	7.5±1.6	1.0,1-3
Tomatine	1	13±3.5	6.2±.81	1.0,0-3	3.7±.85	5.8±1.1	1.0,1-2
	G=1.44 df=1, NS	F=0.41 df=1,17 NS	F=2.09 df=1,16 NS		F=4.01 df=1,17 NS	F=0.75 df=1,17 NS	

[1]Abbreviations as in Table 3.1

Experiment 3

The addition of alkaloids to *S. tuberosum* leaves increased variability of meal size among Alberta beetles (Levene's equal variance test, $F=2.97$, d.f.$=3, 36$; $P=0.045$) but not overall food consumption (Table 3.3). Relative to controls, atropine had the most pronounced effect on feeding behaviour as four of the ten animals tested did not feed (likelihood-ratio test $G=12.5$, d.f.$=3$, $P<0.01$).

Within group variability in pre-feed gustatory sampling increased with the addition of alkaloids (Levene's equal variance test, $F=9.55$; d.f.$=3, 36$; $P<0.0001$). When variances were equalized among treatment groups using square root transformation, ANOVA yielded a significant effect on gustatory sampling ($F=4.29$; d.f.$=1, 36$; $P=0.01$). However, only atropine treated leaves ($F=7.94$, d.f.$=1$, $P<0.01$ using experiment wise error rate) and not tomatine treated leaves ($F=.177$, d.f.$=1$, NS) differed from the control group. Not shown in Table 3.3 is the difference in gustatory sampling time between non-feeders (mean$=11.2$s) and feeders (mean$=6.7$s) in the atropine treatment group.

Both the number of sampling and feeding sites tended to increase with the addition of foreign alkaloids, a pattern similar to that observed with feeding behaviour on marginal host plants (see Chapter 2).

Discussion

The purpose of this investigation was to determine whether solanaceous alkaloids added to plants would induce a sensory-based reduction of feeding in *L. decemlineata* adults. Detailed behavioural analysis of plant sampling and feeding behaviour provide no evidence for such inhibitory effects for the steroidal glycoalkaloids tomatine and solanine, but reveal a clear effect due to the tropane alkaloid atropine.

It has often been implied in the literature that resistance of *L. esculentum* to *L. decemlineata* is due to the action of tomatine on the insect's sensory system (see Bongers 1970, Schoonhoven 1982). However, in the present experiments, increasing the tomatine

Table 3.3. First meal feeding and associated sampling behaviours by beetles from Alberta tested with alkaloid treated potato leaves (concn.=2mM). Sampling data include both feeders and non-feeders if present in treatment group. Values given are means±standard errors or medians, range. N=10 per teatment.

		FEEDING			SAMPLING[1]		
Treatment	#Reject	Amount (mm^2)	Rate (mm^2/min)	#Sites	Exp(s)	GS(s)	#GS Sites
Control	0	31±2.2	6.2±.71	1.0, 1-4	3.3±.62	3.2±.41	1.0, 1-5
Solanine	0	37±5.2	6.0±.75	2.0, 1-3	3.6±.50	2.8±.65	1.0, 1-2
Tomatine	0	31±4.8	5.8±.49	1.5, 1-2	3.3±.59	4.1±1.04	2.0, 1-5
Atropine	4	31±6.1	3.9±.61	3.5, 1-4	3.5±.47	8.4±1.95	4.0, 1-8
	G=12.5** df=3	F=0.45 df=3,32 NS	F=1.83 df=3,32 NS	G=5.80 df=3 NS	F=0.06 df=3,34 NS	F=4.29*[2] df=3,36	G=11.6** df=3

[1] Abbreviations as in Table 3.1.
[2] after square root transformation
* $p<0.05$, ** $p<0.01$

concentration to levels characteristic of "resistant" tomato varieties (Sinden et al. 1978) had no significant effect on the beetles' behaviour that would indicate a chemosensory based mode of action for the alkaloid. The reduced feeding previously reported from long-term experiments with this compound may have been due to post-ingestive effects (e.g., anorexia or intoxication).

Atropine is a non-steroidal alkaloid found in several solanaceous plants not used as hosts by *L. decemlineata*. Kogan (1976) has suggested *L. decemlineata* may be physiologically adapted to steroidal alkaloids but not to tropane alkaloids, thereby allowing expansion of the species' host range to include tomato but not to include *Datura* or *Atropa* species. The strong inhibition of feeding due to atropine tends to support this hypothesis. However, the results must be interpreted with caution. Atropine is not normally found in high concentrations in solanaceous plants. Hyoscyamine, the dominant tropane alkaloid in many *Datura* species, is found in leaves up to 0.4% dry weight, or approximately 1.5mM (Avery et al. 1959). However, this concentration is somewhat less than that used in my experiment.

Also, beetles may reject plants on the basis of olfactory input before proceeding to the gustatory sampling stage where alkaloids are first contacted. Therefore, testing a foreign alkaloid in a primary host may lead to erroneous conclusions. My own results (see Chapter 2) and those of Visser and Ave (1978) have shown that *L. decemlineata* adults are capable of fine, olfactory-based discrimination of plants. Thus, beetles may not be attracted to plants containing tropane alkaloids in the field, and if they do contact them, the beetles may not sample the plant fluids.

For several reasons, interpreting the evidence for secondary plant compounds acting as behavioural deterrents is difficult based on present data (reviews by Chapman 1974, Schoonhoven 1982, and several chapters in Rothensal and Janzen 1979). First, both electrophysiological and behavioural studies have relied largely on the use of pure chemicals tested out of context with natural plant stimuli. Feeding bioassays typically use secondary

compounds incorporated into artificial diets or media such as filter paper with perhaps only one feeding stimulant present. For example, Bernays and Chapman (1977) tested over one-hundred secondary compounds for their effects on feeding in *Locusata migratoria* by incorporating the chemicals in wheat flour discs. As Dethier (1977) has noted, these experiments by design tend to overemphasize the importance of the compound in question because most of the positive sensory input used by the insect to recognize plants is removed. In the above-mentioned study, the authors actually provide data showing that the addition of a single positive stimulus (sugar) to a wafer containing one of their most effective deterrents (tomatine) completely neutralized the feeding inhibition, yet the authors go on to conclude that "the failure to eat most plants results from the presence of one or more chemicals in amounts which inhibit feeding."

Second, most experiments have been conducted on the larval stage, yet it is adults who usually do the host selecting in holometabolous insects. Larvae are generally poorly equipped to seek out new hosts and are therefore usually less discriminating in their feeding. Studies demonstrating negative effects on larval fitness due to secondary compounds may be valid in their own right; however these results should not be intepreted in terms of host selection processes.

Third, many of the compounds which are tested on insects come from plant families never used, or perhaps never even encountered, by the insects in nature. Since those insect species which have been carefully studied are capable of discriminating among plants on the basis of other sensory modalities (vision, smell) in addition to taste (see Chapter 2), the relevance of these kinds of experiments must be questioned.

Wherever possible, future studies using secondary plant compounds should use the insect's natural plant as the feeding substrate; this will avoid biasing the experiments in favor of positive results. Also, ecologically relevant combinations of plants and compounds should be used if results are going to be interpreted in terms of host selection mechanisms and evolutionary processes. The results of the present study suggest the role of alkaloids in

affecting host choice in *L. decemlineata* has been overemphasized by the nature of experimental methods used.

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IV. Regional differences in host plant preference in the Colorado potato beetle and the role of behaviour in insect-plant evolution

Introduction

Many wide ranging species of herbivorous insects are actually composed of regional populations of dietary specialists variously refered to as biotypes, ecotypes or host races.[4] Populations may become geographically isolated on one host plant, and subsequently evolve as obligate monophages; but, species may also develop regional differences in host use where there is no apparent restriction in plant availability (Gilbert 1979, Cates 1981). The adaptive response of a host-adapted race may be physiological, resulting in increased larval digestive efficiency (Rausher 1982, Scriber 1983), or behavioural, leading to altered oviposition preference (Singer 1971, Tabashnik et al. 1981, Jaenike and Grimaldi 1983) or both.

Studies of host-adapted populations of insect herbivores have been prominent in the evolutionary literature for over a century (Walsh 1864, Thorpe 1930, Mayr 1942, Dethier 1954, White 1978). Recently, a number of authors have claimed to document sympatric host race formation and speciation in herbivorous insects (Bush 1975, review by Strong et al. 1984) and have challenged the traditional view of allopatric speciation via geographically isolated populations (Mayr 1963, Dobzhansky 1970, Futuyma and Mayer 1980). Despite their theoretical importance, however, there have been relatively few detailed studies of the properties of insects that facilitate host plant specialization.

Inadvertently, human agricultural practices have given us an opportunity for studying evolutionary interactions between insects and plants in the field. Rapid colonization of introduced crops has been documented for a variety of insects (see reviews by Price and Waldbauer 1982, Gould 1983). A well known example is the Colorado Potato Beetle *L. decemlineata*. Once confined to the western United States and Mexico where it fed primarily on buffalo bur, *Solanum rostratum*, *L. decemlineata* colonized the potato plant (*S.*

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[4] I will follow recent convention and restrict use of the term "host race" to sympatric populations of host adapted insects (Jaenike 1981).

tuberosum) soon after the plant was introduced to the United States, and later dispersed throughout most of North America and Europe with the expansion of potato agriculture (see Tower 1906, and Johnson 1969, for historical summaries). *L. decemlineata* is now reported to use a number of native and introduced plants as hosts, most in the family Solanaceae.

Hsiao(1978) has studied larval adaptations of several geographic populations of *L. decemlineata* in North America, and found local specialization (probably in digestive efficiency) where populations are isolated largely on one plant; for example in Arizona where *S. elaeagnifolium* is the only available host plant. In this study, I report on regional differences in feeding behaviour by adults of the same species from three North American populations in different states of evolutionary transition with respect to host plant use (see Chapter 1).

Adult food choice is often ignored as a factor in insect-plant evolution, with emphasis instead being placed on female oviposition behaviour, larval feeding behaviour and larval digestive physiology. Yet, like many other holometabolous insects, *L.decemlineata* adults require a period of feeding on a suitable host both for the development of ovaries (deWilde and deLoof 1973) and wing muscles (see reviews by Johnson 1969, 1974). Subsequent migration or dispersal may occur, but only by females with incompletely developed ovaries (gravid females do not fly) which then undergo a period of maturation feeding prior to egg-laying. For these species, the distribution of females on plants, and therefore their oviposition sites, is directed largely by adult food choice.

Laboratory oviposition studies have shown that *L. decemlineata* females lay large numbers of eggs on non-host plants that are toxic to developing larvae (Bongers 1970, Hsiao and Fraenkel 1968). However, these studies are probably meaningless, since having no food preference for these plants, the ovipositing female will not normally settle on these plants in nature. Bongers (1970, p.63) concludes "... oviposition normally will occur on the preferred food plant on which the females complete their maturation feeding. It is, therefore not to be expected that oviposition preference will interfere with food preference". In fact,

L.decemlineata may not have an "oviposition preference" at all in terms of a discrete behavioural mechanism; eggs are simply deposited where the gravid female settles to feed.

In this paper, adult feeding preference will be considered crucial in determining patterns of host plant use by *L. decemlineata*. New data on regional differences in feeding behaviour are reported and discussed in relation to a wealth of information currently available for this species. Specifically, I compare and contrast the evolutionary response of larval fitness (physiological) and adult behavioural (host selection) characteristics and assess the relative contributions of each in facilitating the host range expansion that has occurred in this insect.

It has been suggested by Futuyma (1983) that genetically labile host selection behaviour may be responsible for mediating rapid host shifts in phytophagous insects. However, I conclude that for *L. decemlineata*, colonization of new hosts has been aided by the broad physiological tolerances of the larvae and their rapid evolutionary response to new host use. Adult feeding behaviour, rather than promoting host transfers, appears to act as a conservative force in the absence of ecological constraints on primary host availability. Previous studies are reviewed which support this conclusion for several other species of insect herbivores.

Materials and Methods

Insects used in this experiment were collected in the field and reared in the laboratory on their respective regional host plant (see Chapter 1, Study organisms). All were first day, naive (no feeding experience) adults.

Feeding behaviour was recorded from a total of one-hundred and forty-four beetles, forty-eight from each geographic population. From these, an equal number were randomly assigned to each of four plant groups, *S. tuberosum* ("Norland" variety), *S. rostratum*, *S. elaeagnifolium*, or *L. esculentum* ("Earliana" variety).

Each insect was tested individually with a separate leaf using a simple apparatus designed to allow videotape monitoring of behavioural events associated with feeding (see Chapter 2 for details). Beetles were allowed free access to a leaf until three minutes without feeding elapsed. This period was previously determined as a minimum inter-meal interval time for this insect. Therefore, unlike conventional studies, this experiment was designed to measure only first meal consumption to avoid possible long term effects on feeding from induction of food preference. However, what I have defined as first feeding bouts are actually quite long feeding episodes that may take over thirty minutes to complete. This is ample time for sensory mechanisms regulating food intake to operate.

Further details of the methods used in this experiment are given in Chapter 2.

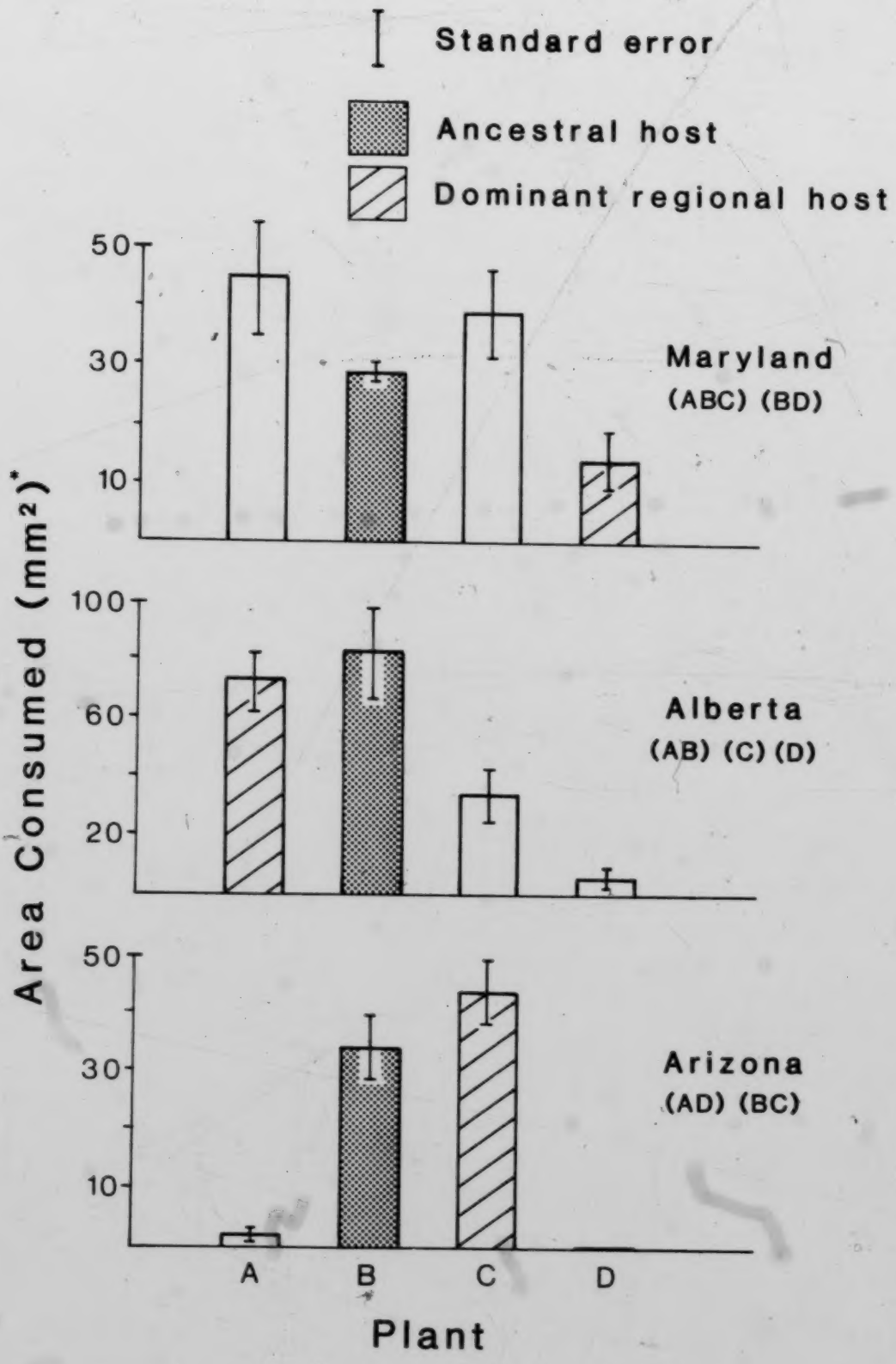
Statistical analysis

This experiment was originally outlined as a two-way factorial design (Population X Plant). However, analysis of the feeding rates of each population (reported in Chapter 2) revealed inherent differences in feeding under laboratory conditions regardless of plant type (Alberta beetles fed on average twice as fast and consumed approximately twice as much as beetles from Arizona and Maryland). Therefore, between population comparisons of food consumption were abandoned, and each population was treated separately for within group differences by Kruskal-Wallis tests and non-parametric multiple comparisons (Conover 1980). Between population comparisons of rejection and acceptance patterns, which should not have been affected by a differential response to laboratory conditions, were treated by contingency table analysis.

Results

Patterns of host plant acceptance and first meal consumption varied considerably among the three geographic *L. decemlineata* populations (Fig 4.1). Only *S. rostratum*, the ancestral host, was accepted for feeding by all beetles regardless of their origin. Beetles from

Figure 4.1. First meal consumption by naive, first-day beetles from three geographic populations. Means enclosed by parenthesis are not significantly different at p=0.05. Plant A=*S. tuberosum* (potato); B=*S. rostratum*; C=*S. elaeagnifolium*; D=*L. esculentum* (tomato). N=48 per population. *Area corrected for differences in leaf thickness.


Standard error
Ancestral host
Dominant regional host
Area Consumed (mm²)
50
30
10
Maryland
(ABC) (BD)
100
60
20
Alberta
(AB) (C) (D)
50
30
10
Arizona
(AD) (BC)
A
B
C
D
Plant

Arizona preferred *S. rostratum* and their regional host plant *S. elaeagnifolium* to *S. tuberosum* and *L. esculentum* ($H=38.1$, d.f.$=3$, $P<0.001$) which were largely rejected (only two of twelve beetles fed on potato, while one nibbled briefly on tomato). Similarly, the Alberta population prefered *S. rostratum* and their most abundant regional host *S. tuberosum* to *S. elaeagnifolium* which in turn was eaten more than *L. esculentum* ($H=21.1$, d.f.$=3$, $P<0.01$).

Despite being reared on *L. esculentum* as larvae, fewer adult beetles from Maryland fed on this plant than the two preferred hosts *S. tuberosum* and *S. elaeagnifolium* ($X^2=4.8$, d.f.$=1$, $P<0.05$), resulting in significantly lower food consumption ($H=10.9$, d.f.$=3$, $P<0.05$). Feeding on *S. rostratum* by Maryland beetles was intermediate, and did not differ statistically from any other plant by pair-wise comparisons.

Discussion

The comparative analysis of feeding using naive adult beetles from several geographic populations provides a clear example of regional differences in host preference with an apparent genetic basis. With regard to evolution of host choice in *L. decemlineata*, there are three important points to be derived from this study: (1) Initial host acceptance by adults is relatively conservative or "fine-tuned". The almost total rejection of *S. tuberosum* by Arizona beetles was unexpected given the close taxonomic affinity of this plant to both *S. elaeagnifolium* and *S. rostratum* within the genus *Solanum* (Whalen 1979, and references therein). (2) Colonization of a new host has not been associated with absolute shifts in feeding preference. Instead, new plants are added to the insects' repertoire of acceptable species as populations evolve in association with them. Thus *S. rostratum*, the ancestral host, is still preferred by more recently evolved populations which have not used the plant locally for at least one-hundred and fifty generations (see Chapter 1). (3) There may be a lag time in the evolution of behavioural mechanism(s) regulating acceptance of novel hosts. Although this insect has recently reached "pest" status on *L. esculentum* in Maryland, beetles from that

area ranked the plant fourth of the four solanaceous hosts tested in this study. One-third of the beetles rejected the plant outright on first contact.

The implication of these observations is that constraints on the availability of primary hosts should be crucial in allowing colonization of new plants. If that is true, one would not predict multiple host use and population differentiation in areas where the ancestral host is common. My own observations and those of Neck (1983) and Hsiao (1978) in the Southwestern United States confirm this prediction. *L. decemlineata* has been recorded from this area for several hundred years (Tower 1906) and *S. rostratum* is still the most frequently used host plant by far, despite the local presence of several *Solanum* species, including *S. elaeagnifolium*. Directional selection for new host use has only been effective in this species at the periphery of its range where some degree of isolation on one plant (Arizona) or one very dominant local plant (Maryland, Alberta) has existed. Under these allopatric conditions, conservative host selection behaviour by adults has not prevented colonization events simply because there has been no choice available but to use the marginal plant.

The role of behaviour in insect-plant evolution

Distinctions are often made in the literature between larval fitness traits (metabolic efficiency) and adult host selection characteristics (usually oviposition) with respect to their relative contributions in promoting phytophagous insect diversity. Some authors downplay the role of physiological specialization and argue that genetically labile behavioural mechanisms promote insect diversity by mediating rapid host shifts (for example, see Futuyma 1983, Futuyma et al. 1984).

The data for *L. decemlineata* do not support this hypothesis. While complete acceptance of *L. esculentum* by Maryland beetles has not yet evolved (see above), larvae reared in the laboratory on this plant show no apparent increase in mortality or protracted development time (Harrison, unpublished data). In addition, Hsiao (1978) has demonstrated that larvae from populations in Arizona and Utah (equivalent to the Alberta insects used in

this study) are physiologically more efficient on their regional hosts than other plants (including *S. rostratum*), whereas adults from these populations show no corresponding degree of behavioural preference for their new hosts. Colonization of novel plants by *L. decemlineata* appears to first involve physiological adaptation (which may lead to physiological specialization) followed in time by the evolution of behavioural mechanisms regulating host selection in adults.

Is this scenario for *L. decemlineata* evolution a special case, or is it true for other phytophagous insect species? Table 4.1 lists all the examples I could find for which both larval fitness and adult host selection traits have been studied within the same species of sexually reproducing insect. A similar table, including many examples of parthenogenetic species of crop pests, was compiled recently by Gould (1983, pp. 627-630). Note that with the exception of Wasserman and Futuyma (1981) (who used an insect that was reared in the laboratory for 300 generations and who did not measure oviposition directly) there are no examples (nor are there in Gould's table) where genetic differentiation of behavioural traits have evolved independently of metabolic changes. There are, however, several examples where metabolic efficiency for a new host has evolved without a concomitant change in oviposition or feeding behaviour, including those species in which the characters were measured soon after the host shift ocurred (i.e. *C. philodice*, *M. destructor*, and *L. decemlineata*).

On theoretical grounds, these results are not suprising. A related question to how much genetic variability is resident within populations for a given trait, is how complex the traits are in terms of their organization. Phytophagous insects are infamous for their abilities to rapidly adapt (physiologically) to resistant crop strains (Maxwell and Jennings 1980) and to insecticides (Georghiou and Saito 1983) and some of these adaptations have been traced to single gene effects (see Gould 1983). In contrast, a new consensus is developing that mechanisms underlying host selection processes in insects are not simple, but complex and interactive (see Chapter Two). This complexity might account for the relatively

Table 4.1. Comparison of between population variability in behavioural and physiological traits with a reported genetic basis. Included are studies in which both types of characteristics have been measured on the same sexually reproducing insect species.

		Characters measured and genetic variability found? (Yes/No)		
Insect	Hosts tested	Behavioural	Physiological	Reference
Colias philodice (Lepidoptera)	wild and domestic Leguminosae	oviposition preference (No)	larval Gr,DT,Sv (Yes) larval PW (No)	Tabashnik (1983)
Euphydryas editha (Lepidoptera)	Scrophulariaceae Plantaginaceae	oviposition preference (Yes)	larval Gr,GE,Sv (Yes)	Singer (1971), Rausher (1982)
Deloyala guttata (Coleoptera)	Convolvulaceae	oviposition preference (No)	larval DT,PW (No) fecundity (Yes)	Rausher (1983), Rausher (1984)
Mayetiola destructor (Diptera)	varieties of wheat	oviposition preference (No)	larval Sv (Yes)	review by Gould (1983)
C. maculatus (Coleoptera)	azuki beans, pigeon peas	oviposition preference (Yes)[1]	larval DT,Sv (No)	Wasserman and Futuyma (1981)
L. decemlineata (Coleoptera)	wild and domestic Solanaceae	adult feeding preference (Y/N)[2]	larval PW,Sv (Yes)	Hsiao (1978), this study

DT=development time; GE=growth efficiency; Gr=growth; PW=pupal weight; Sv=survivorship
[1]measured indirectly as part of a complex trait called "realized fecundity/oviposition preference"
[2]Three populations tested:two have evolved feeding preference to match ancestral host, a third has not; however, anecdotal evidence suggests larvae from this population are physiologically adapted.

slow evolution of behavioural traits indicated by present data.

One of the basic assumptions of theories of sympatric speciation is that simple genetic changes can mediate rapid changes in host preference (see Bush 1975). This assumption may have been influenced by the conventional belief that mechanisms of host recognition in insects are simple, and by the traditional view that shifts into new adaptive zones or niches are initiated by behaviour (Mayr 1963). With regard to insect-plant evolution, both these premises deserve re-evaluation. First, as stated above, there is growing evidence that mechanisms underlying host choice in insects are complex, and relatively slow to respond to evolutionary changes in host use. There is no convincing example in the literature of a gene mutation spontaneously giving rise to a new population of host-adapted genotypes. This does not mean that such an event is impossible, but it has yet to be demonstrated.

Second, the idea that changes in behaviour ultimately drive colonization events (see Futuyma 1983, Parsons 1983) perhaps has been applied too loosely to phytophagous insects. The majority of these insects can rightly be considered parasitic (Price 1980), and most are highly specialized in terms of their physiological adaptation to plants (see Introduction). Unlike non-parasitic organisms whose foraging tactics may uncover new resources or habitats, changes in host (i.e. habitat) use by insects may result in a significant loss of fitness through decreased fecundity or larval mortality. Therefore, if other factors are not considered, evolutionary theory would predict selection against, not for, lability of host selection behaviour.

A reconsideration of the role of behaviour as potentially representing a conservative force in insect-plant evolution affects the way we percieve causation in these systems. Frequent references in the literature to behavioural changes initiating, or causing host shifts implies a role for behaviour not substantiated by present data. Fox and Morrow (1981) have warned that host plant specialization may not be an inherent property of insects, but may be imposed by a complex of ecological factors in the insects' local environment including

microclimate, variable host phenology, presence of predators, parasites, and so on. If there are "causes" of host specialization and host shifts in phytophagous insects, they may lie in the varied nature of these selection regimes, as well as in properties intrinsic to the organisms.

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V. Concluding Discussion

In a recent review of the mechanisms of host plant recognition in insects, Dethier (1982) warned that much of the evidence supporting a dominant role for secondary plant compounds was correlative. Stressing the need for demonstrating causal relationships, the author noted "Since the acceptance or rejection of a plant depends in the final analysis on the ability of the herbivore to assess some characteristics of the plant, the fundamental question is whether the compounds under discussion affect individual behavior in any way."

In this study, I used an analysis of individual insect behaviour to investigate the sensory basis of host recognition in adult Colorado potato beetles, *L. decemlineata*. The results indicate that these beetles are capable of graded discrimination of potential host plants, perhaps based on their total "Gestalt" or flavour. Moreover, variable acceptance of plants among regional populations of insects is not related to the presence of glycoalkaloids acting as feeding deterrents. For this species, it appears that the primary constraint on host acceptability is set by the beetles' finely tuned sensory system which functions independent of secondary plant compounds.

For this reason, it is unlikely that non-steroidal alkaloids (e.g. atropine) have been a major factor in preventing colonization of plants in more distant Solanaceae genera, since the beetles are probably not attracted to these plants in the field. Theoretically, if *L. decemlineata* were to continue with a host range expansion such that individual beetles developed generalist feeding habits, then certain plants containing behavioural deterrents would eventually undergo attempted colonization. However, it appears that regional populations of *L. decemlineata* are beginning to evolve behavioural specificity for their new hosts — a pattern typical of many phytophagous species. Within these differentiating populations, choice of plants will continue to be constrained by sensory systems tuned to the total chemical make-up of their hosts. This may effectively limit future host range expansion by *L. decemlineata* to plants with chemical profiles similar to those of *Solanum* species, except where agricultural practices induce selection for more distantly related plants

(as in Maryland, on tomato).

Clearly, other factors are shaping behavioural specificity in *L. decemlineata* besides secondary plant compounds. Future research will be needed to determine how widespread this phenomenon is in plant feeding insects, but it is unlikely that *L. decemlineata* represents a special case. Most insects probably choose plants on the basis of complex patterns of sensory information gathered across several sensory modalities including smell, taste, and vision (Dethier 1982, Miller and Strickler 1984); an ability which endows them with the potential for perceiving, and responding to, subtle variations in plant quality. While many of these interactions involve secondary compounds, it is becoming clear that these chemicals rarely act as single, dominant factors in regulating host choice behaviour. Even among Cruciferae feeding insects, glucosinolates acting as stimulants are not entirely responsible for host discrimination as was originally supposed (Nielsen et al. 1979, Chew 1980, Feeny et al. 1983).

Perception of host plant quality based on variations in complex patterns of sensory stimuli is a fundamentally different concept than single compound, single neural pathway models (see Chapter two). The existence of an intervening control mechanism in the CNS which acts to integrate sensory input both within and across sensory modalities, and whose own state (and therefore outputs) is modifiable through ontogeny, has been almost totally ignored in classic input-output studies of insect feeding behaviour which treated the organism as a "black box" (see, for example, Blom 1978).

Attempts are now being made, however, to incorporate CNS mechanisms in models of host plant discrimination in insects. For example, Rausher (1983) has borrowed the sensory template concept originally developed to account for species-specific responses to auditory communications (Marler 1976, Hoy et al. 1977). But, sensory templates are assumed to represent precise neural networks in the brain which respond only to very specific patterns of sensory input. Application of this concept to plant perception by insects becomes awkward, because it leads to postulating separate templates for each individual plant species

an insect might encounter. It is perhaps more economical (in terms of nervous system function) to think of a single CNS reference state (probably represented by neural networks) whose characteristics are determined genetically, but are modifiable by experience. Schoonhoven's (1977) analogy of a lock and key mechanism is useful in this context — the lock representing the CNS reference state and the key, patterns of sensory input. Individual insects that have broader acceptance thresholds for marginal host plants may have a "broadly tuned" lock and key mechanism. Selection for these animals within colonizing populations of insects may represent a first step in the evolution of new insect-plant associations.

At this time it is not clear how mechanisms underlying host selection behaviour affect the evolution of feeding preferences in insects. Current opinion seems to favour an active role for behaviour based on the assumption that it may be genetically labile (Bush 1975, Futuyma 1983, Futuyma et al. 1984). However, in this study I present evidence that indicates host selection behaviour may be a conservative trait in insect-plant evolution (see Chapter Four). Although data are limited, behavioural adaptations in some species appear to evolve slowly during colonization events; an observation that may be attributed to the complexity of the sensory systems involved (see above). Unfortunately, we know virtually nothing about the genetic basis of host selection in insects. But, it would be surprising if only single loci were involved, since even relatively simple behaviour patterns in animals usually have a polygenic basis (Frank 1974).

For insect species with conservative host selection behaviour, the importance of local selection pressures in maintaining altered patterns of host use over successive generations cannot be overstated. Phytophagous insects harbour considerable within population genetic variability for both physiological and behavioural traits related to host use (see review by Gould 1983, and recent papers by Jaenike and Grimaldi 1983, Via 1984). Therefore, all but strictly monophagous species are to some degree preadapted for feeding on other plant species. Directional selection for using these novel hosts will depend on the nature of local selection regimes (relative plant abundance, variable host phenology, microclimate, soil

conditions and so on; see Fox and Morrow 1981) which can affect both the probability of ovipostion on a given plant and the viability of developing larvae.

During the recent host range expansion by *L. decemlineata*, some degree of isolation on a dominant regional host appears to have been critical in allowing local specialization to develop. Given strong enough selection pressures however, populations may diverge on different plants in close proximity and in spite of some gene flow (Ehrlich and Raven 1969, Endler 1977, Templeton 1981). The potential for ecological factors to interact in unique ways to affect patterns of host plant use by insects stresses the need for future studies to examine in detail the behaviour and ecology of individual species at the population level.

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VI. Appendix I: Beetle cultures

Beetle larvae were reared in 6 liter glass aquaria containing approximately 2cm of gravel, covered by 8cm of loose sand. A glass tube inserted through the sand and into the gravel layer allowed adding water as needed to provide a moisture gradient for pupating larvae. Adult colonies were reared in larger (68 liter) aquaria prepared in a similar fashion. All insects were maintained at 16L:8D, 25°C±3° under a combination flourescent and incandescent light fixture.

Fresh food was provided as needed in the form of potted plants or leaves placed in water. Egg batches were collected from adult colonies and stored in petri dishes until they hatched. Typically, larvae were kept in these dishes with food before being transfered to aquaria at the second instar stage. At room temperature, larvae passed through four instars and pupated in about three weeks. Pupation time averaged seven to ten days.

By rotating larvae between two or more aquaria and timing the emergence of adults, beetles with no feeding experience as adults were available for experiments.

VII. Appendix II: Plant growth conditions

Plants were grown year round in a heated, outdoor greenhouse. Daylength was maintained at a minimum of 16 hours using flourescent fixtures or Sonline high-intensity sodium lamps as supplemental lighting. Nightime temperatures were kept at about 15°C. Daytime temperatures in the greenhouse varied from 20°C to 30°C.

Potato plants ("Norland" variety) were grown from tubers seeded in a large bed. All other plants including tomato ("Earliana" variety) were grown from seed. Germination of *S. elaeagnifolium* and *S. rostratum* seeds (which possess a hard seed coat) was improved by soaking the seeds for twenty-four hours in 2000ppm gibberellic acid before planting. Standard soil mixtures were used for all plants. Plants were fed regularly (once a week in summer, once a month in winter) with 20-20-20 or 28-14-14 fertilizer.

Since one of the objectives of this study was to record sequences of insect behaviour on unaltered plants, no insecticides were used which might have left detectable residues on the plant surface. Greenhouse pests were controlled by pruning infested plants, washing them with water, or applying soap solutions. To remove any soap residue, plants were routinely washed with water before being used in experiments or as culture food.

VIII. Appendix III: Total glycoalkaloid levels (TGA) and percent water content from leaves of four solanaceous host plants of *Leptinotarsa decemlineata*

Plant	% Water[1]	TGA[2](mg/g dry wt)
S. tuberosum	91	5.2
S. rostratum	84	0.53
S. elaeagnifolium	86	0.85
L. esculentum	88	7.8[3]

[1]Calculated by subtracting dry weight from wet weight. Dry weight determined by oven drying ten leaves at 100°C for twenty-four hours.
[2]Following methods of Fitzpatrick and Osman (1974), and Fitzpatrick et al. (1978).
[3]from Sinden et al. (1978); flowering plants (60 days) grown under long days (16 hr).